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(71) Applicant *(for all designated States except US)*: ADVANCED MEDICINE, INC. [US/US]; 280 Utah Avenue, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MORAN, Edmund, J. [CA/US]; 131 Chaves, San Francisco, CA 94127 (US). NUMEROF, Robert, P. [US/US]; Apartment 4, 140 Graystone Terrace, San Francisco, CA 94114 (US). GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US).

(74) Agents: SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).

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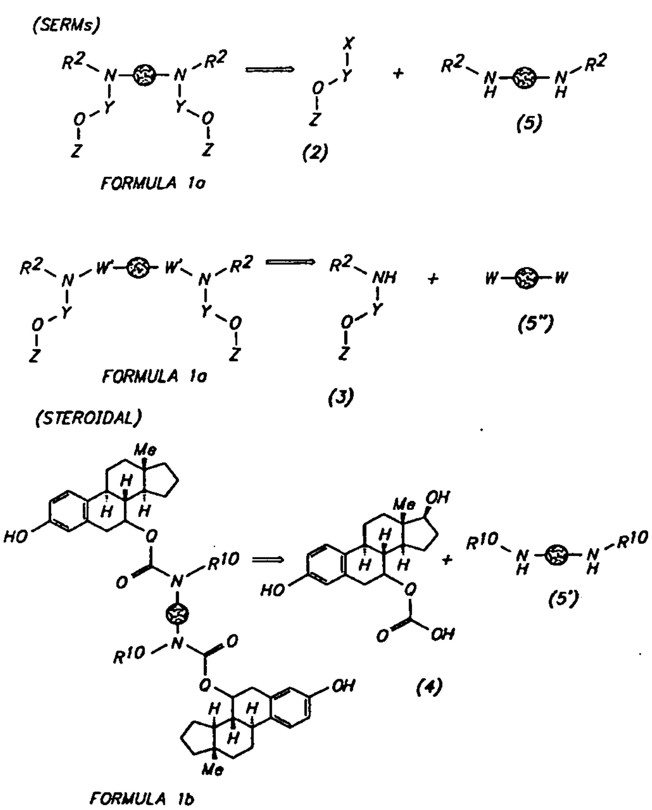
(54) Title: NOVEL THERAPEUTIC AGENTS THAT MODULATE ESTROGEN RECEPTORS

(57) Abstract

Disclosed are novel multi-binding compounds (agents) which bind estrogen receptors. The compounds of this invention comprise a plurality of ligands each of which can bind to such receptors thereby modulating the biological processes/functions thereof. Each of the ligands is covalently attached to a linker or linkers which may be the same or different to provide for the multi-binding compound. The linker is selected such that the multi-binding compound so constructed demonstrates increased modulation of the biological processes mediated by the estrogen receptors.



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NOVEL THERAPEUTIC AGENTS THAT MODULATE ESTROGEN RECEPTORS

Cross Reference to Related Applications

This application claims the benefit of U.S. Provisional Application Serial Numbers 60/088,466, filed June 8, 1998, and 60/092,938, filed July 15, 1998, both of which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to novel therapeutic agents which bind to mammalian receptors and modulate their activity. More particularly, the invention relates to novel therapeutic agents that bind to and modulate the *in vivo* activity of estrogen receptors in mammals by acting as multi-binding compounds. The therapeutic agents or multi-binding compounds described herein comprise at least two ligands connected by a linker or linkers, wherein said ligands in their monovalent state bind to and/or are capable of modulating the activity of the estrogen receptor. The linking moiety is chosen such that the multi-binding compounds so constructed demonstrate increased biological activity as compared to individual units of the ligand. The invention also relates to methods of using such compounds, to methods of preparing such compounds and to pharmaceutical compositions containing them.

These multi-binding compounds are particularly useful in treating mammalian conditions that are mediated by the estrogen receptors targeted by the ligands, such as breast cancer, osteoporosis and atherosclerosis. Accordingly, this invention also relates to pharmaceutical compositions comprising a

pharmaceutically acceptable excipient and an effective amount of a multi-binding compound of this invention.

Additionally, the multi-binding compounds are useful as affinity resins for affinity chromatography. When so employed, the compounds of the invention may be used as a tool in immunoprecipitation. The compounds may be used to identify a receptor *in vitro* for example in microscopy, electrophoresis and chromatography.

References

The following publications are cited in this application as superscript numbers:

1. J. March, *Advanced Organic Chemistry, 4th Edition*, Wiley-Interscience New York (1992);

2. *Remington's Pharmaceutical Sciences,* Mace Publishing Company, Philadelphia, PA, 17th ed. (1985);

3. Green, *Protective Groups in Organic Synthesis*, 2nd Edition, John Wiley & Sons, New York, New York (1991);

4. Bergmann et al., "Bivalent Ligands as Probes of Estrogen Receptor Action," *J. Steroid Biochem. Molec. Biol.* (1994) **49(2/3)**:139-152;

5. Gradishar et al., "Clinical Potential of New Antiestrogens," *J. of Clin. Onco.* (1997) **15(2)**:840-852;

6. Grainger et al., "Tamoxifen: Teaching an Old Drug New Tricks?" *Nature Medicine* (1996) **2(4)**:381-385;

7. Katzenellenbogen et al., "Antiestrogens: Mechanisms of Action and Resistance in Breast Cancer," *Canc. Res. Treat.* (1997) **44**:23-38;

8. Willson et al. "Dissection of the Molecular Mechanism of Action of GW5638, a Novel Estrogen Receptor Ligand, Provides Insights into the Role of Estrogen Receptor in Bone," *Endocrinology* (1997) **138**:3901-3911;

9. Evans et al. "Tissue-Selective Action of Estrogen Analogs," *Bone* (1995) **17(4, supp)**:181S-190S;

10. Ke et al. "Comparative Effects of Droloxifene, Tamoxifen and Estrogen on Bone, Serum Cholesterol and Uterine Histology in the Ovariectomized Rat Model," *Bone* (1997) **20(1)**:31-39;

All of the above publications are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

A receptor is a biological structure with one or more binding domains that reversibly complexes with one or more ligands, where that complexation has biological consequences.

Receptors can exist entirely outside the cell (extracellular receptors), within the cell membrane (but presenting sections of the receptor to the extracellular milieu and cytosol), or entirely within the cell (intracellular receptors). They may also function independently of a cell (e.g., clot formation). Receptors within the cell membrane allow a cell to communicate with the space outside of its boundaries (i.e., signaling) as well as to function in the transport of molecules and ions into and out of the cell.

A ligand is a binding partner for a specific receptor or family of receptors. A ligand may be the endogenous ligand for the receptor or alternatively may be a synthetic ligand for the receptor such as a drug, a drug candidate or a pharmacological tool.

The ligands that bind to cellular receptors may be specifically classified as follows:

1) Full agonists - ligands that when bound trigger the maximum activity seen by natural ligands;

2) Partial agonists- ligands that when bound trigger sub-maximal activity;

3) Antagonist- ligands that when bound inhibit or prevent the activity arising from a natural ligand binding to the receptor. Antagonists may be of the surmountable class (results in the parallel displacement of the dose-response curve of the agonist to the right in a dose dependent fashion without reducing the maximal response for the agonist) or insurmountable class (results in depression of the maximal response for a given agonist with or without the parallel shift);

4) Inverse antagonist-ligands that when bound decrease the basal activity of the unbound receptor (if any).

There are four fundamental measurable properties that pertain to the interaction of a ligand with its receptor:

1) the affinity of the ligand for the receptor, which relates to the energetics of the binding;

2) the efficacy of the ligand for the receptor, which relates to the functional downstream activity of the ligand;

3) the kinetics of the ligand for the receptor, which defines the onset of action and the duration of action; and

4) the desensitization of the receptor for the ligand.

With regard to the ligand, it is the combination of these properties that provides the foundation for defining the nature of the functional response. Thus, an activating ligand (or agonist) has affinity for the receptor and downstream efficacy. In contrast, an inhibiting ligand (antagonist) has affinity for the receptor but no efficacy.

Selectivity defines the ratios of affinities or the ratios of efficacies of a given ligand compared across two receptors. It is the selectivity of a specific drug that provides the required biological profile.

Current drugs (ligands) targeting receptors have clinical shortcomings identified by one or more of low efficacy, low affinity, poor safety profile, lack of selectivity or overselectivity for the intended receptor, suboptimal duration of action and onset of action, and development of resistance or desensitization of the receptor for the ligand. Accordingly, it would be beneficial to develop ligands that have improved affinity, efficacy, selectivity, onset of action and duration of action, and that delay or circumvent the onset of resistance or desensitization.

Affinity of ligand for target receptor

An increase in ligand affinity to the target receptor may contribute to reducing the dose of ligand required to induce the desired therapeutic effect. A reduction in ligand affinity will remove activity and may contribute to the selectivity profile for a ligand.

Efficacy of ligand at a target receptor (functional effect)

An increased ligand efficacy at a target receptor can lead to a reduction in the dose required to mediate the desired therapeutic effect. This increase in efficacy may arise from an improved positive functional response of the ligand or a change from a partial to full agonist profile. Reduced efficacy of a full agonist to a partial agonist may provide clinical benefit by modulating the biological response.

Selectivity of ligand compared across receptor subtypes

An increase in the selectivity of the ligand across receptor subtypes requires that the affinity or efficacy of the ligand at other receptors is reduced relative to the desired receptor. A decrease in the selectivity of the ligand may also be desired.

Onset of Action

More rapid onset of action of the ligand to effect a biological response is often preferred.

Duration of Action

An increased duration of action of the ligand to effect a biological response may be preferred.

Desensitization of the receptor for the ligand

Desensitization, or development of resistance, is best defined as the variety of processes by which the functional interaction of the receptor with its ligand are influenced. These processes lead ultimately to a reduction in cellular response to the activating agonist. Such phenomena are most often observed during prolonged stimulation of the receptor. The two main pathways for receptor desensitization are reduction in receptor density or changes in receptor structure by phosphorylation mechanisms. For example, resistance to tamoxifen in the treatment of breast cancer sometimes develops.

Alternatively, receptor desensitization may occur through changes in receptor structure, such as receptor phosphorylation.

Receptor oligomerization also plays a role in receptor function. It is also known that dimerization is involved in the functioning of the steroid receptor, such as an estrogen receptor.

The estrogen receptor ("ER") is a member of the nuclear receptor superfamily of transcription factors[10] which belongs to the superfamily of steroid/thyroid hormone receptors. Other members of this family include glucocorticoids receptors, mineralocortocoid receptors, receptors for retinoic acid, 9-*cis* retinoic acid, thyroid hormone and vitamin D, and the like. At least two different estrogen receptors (i.e., ER-α and ER-β) have been identified which

have different tissue distribution *in vivo* (e.g., ER-α is found in higher concentrations in the uterus and pituitary wherase ER-β is found in higher concentrations in the ovary, prostate, lung, intestine and bladder). In cells expressing multiple forms of the receptor, heterodimers can be formed.

In the absence of estrogen, the estrogen receptors resides in the nucleus of target cells in a transcriptionally inactive state.[10] Upon binding ligand, ER undergoes a conformational change, initiating a cascade of events leading ultimately to its association with specific regulatory regions within target genes.[10] The ensuing effect on transcription is influenced by the cell and promoter context of the DNA-bound receptor.[10]

Stimulation of the estrogen receptor by estrogen has been correlated to *in vivo* activities such as:

A. regulation of growth, differentiation, and functioning of many reproductive tissues including the uterus, vagina, ovary, oviduct and mammary glands;

B. bone maintenance including stimulation of differentiation and activity of osteoblasts and inhibition of activity of osteoblasts;

C. cardioprotective effects including increasing high density lipoprotein (HDL) and decreasing low density lipoprotein (LDL);

D. modulating certain brain functions including mood, reproductive behavior, learning and memory; and

E. proliferation of breast cancer cells including production of growth factors or potentiation of responses to growth factors and/or induction of progesterone receptor expression.

In view of the above, novel ligands (drugs) for the estrogen receptor having selective antagonist and/or agonist effects would be particularly desirable in order to inhibit or treat disease conditions associated therewith (e.g., antagonist behavoir for inhibiting or treating breast cancer and agonist behavior

for inhibiting or treating osteoporosis). Such novel ligands would preferably achieve the desired potency and therapeutic effect by modulating one or more of the ligand's properties as to efficacy, affinity, safety profile, tissue selectivity, duration of action and/or onset of action. Additionally, the novel ligands would preferably also circumvent or delay the onset of resistance or desensitization.

SUMMARY OF THE INVENTION

This invention is directed, in part, to novel multi-binding compounds that bind estrogen receptors and consequently these compounds can be used to treat conditions mediated by estrogen receptors such as breast cancer, osteoporosis and atherosclerosis.

Accordingly, in one of its composition aspects, this invention is directed to a multi-binding compound and salts thereof comprising 2 to 10 ligands, which may be the same or different and which are covalently attached to a linker or linkers which may be the same of different, wherein each of said ligands comprises a ligand domain capable of binding to an estrogen receptor and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

The multi-binding compounds of this invention are preferably represented by formula I:

$$(L)_p(X)_q \qquad \text{I}$$

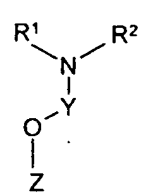
wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20; and pharmaceutically acceptable salts thereof, and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not hexestrol in the erythro configuration.

Preferably, q is less than p.

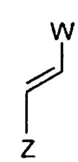
In one embodiment of this invention, each of the ligands comprises a ligand domain capable of binding to an estrogen receptor which ligands exhibit antagonistic behavior. In another embodiment of this invention, each of the ligands comprises a ligand domain capable of binding to an estrogen receptor which ligands exhibit agonistic behavior. In still another embodiment of this invention, at least one of these ligands exhibits agonistic behavior and at least one of these ligands exhibits antagonist behavior.

In another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound, or a pharmaceutically acceptable salt thereof, comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, wherein each of said ligands comprising a ligand domain capable of binding to one or more estrogen receptors and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

Preferred ligands include those comprising a ligand domain capable of binding to one or more estrogen receptors which ligands possess both ER agonist and antagonist behavior. Such preferred ligands include, by way of example, compounds of the formula:



or

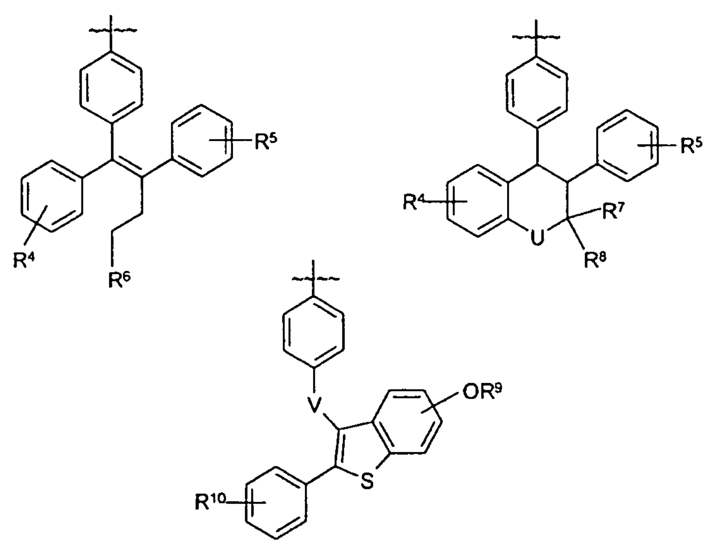


where R^1 and R^2 are selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, or are joined to form, together with the nitrogen atom to which they are pendent, a heterocyclic group;

W is selected from the group consisting of -COOH and -COOR^3 where R^3 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl and heterocyclic;

Y is selected from the group consisting of alkylene, alkenylene, substituted alkylene and substituted alkenylene,

Z is selected from the group consisting of:



where R^4 is selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, aryl, aryloxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxy, hydrogen, halogen, heteroaryl, heteroaryl, heterocyclic, -OP(O)$(OH)_2$ and -OSO_3H;

R^5 is selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^6 is selected from the group consisting of hydrogen, hydroxyl, halogen and cyano;

R^7, R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^{10} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

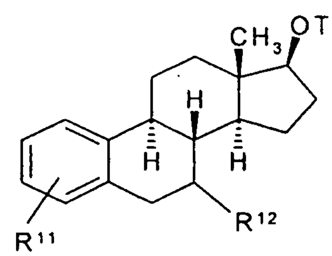
U is selected from the group consisting of methylene, ethylene, -O- and -S-; and

V is selected from the group consisting of a covalent bond, -C(O)- and -C(S)-;

with the proviso that one of R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} links the ligand to a linker;

and pharmaceutically acceptable salts thereof.

In another preferred embodiment, the ligand comprises a ligand domain capable of binding to one or more estrogen receptors which ligand possess ER agonist. Such preferred ligands include, by way of example, compounds of the formula:



where R^{11} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

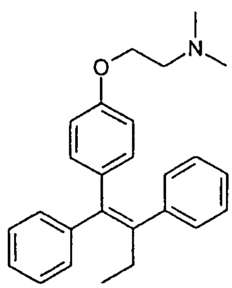
R^{12} is selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

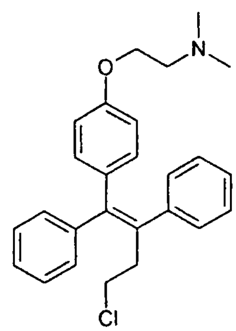
T is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, cycloalkyl, substituted cycloalkyl, heteroaryl and heterocyclic

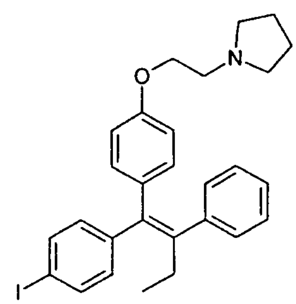
with the proviso that at least one of R^{11}, R^{12} and T links the ligand to a linker;

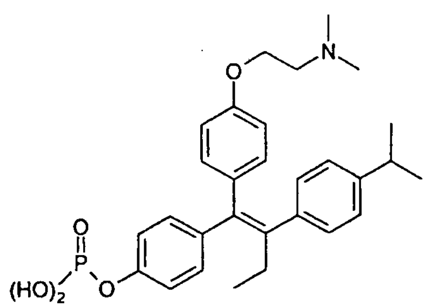
and pharmaceutically acceptable salts thereof.

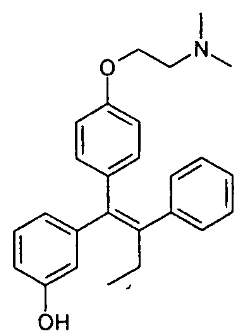
Such ligands include those selected from the group consisting of:

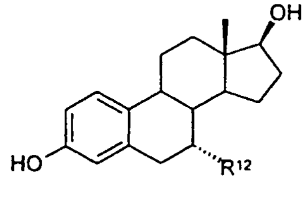






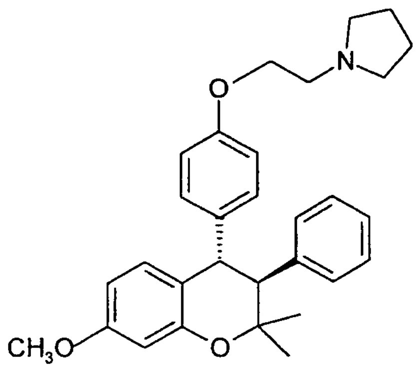


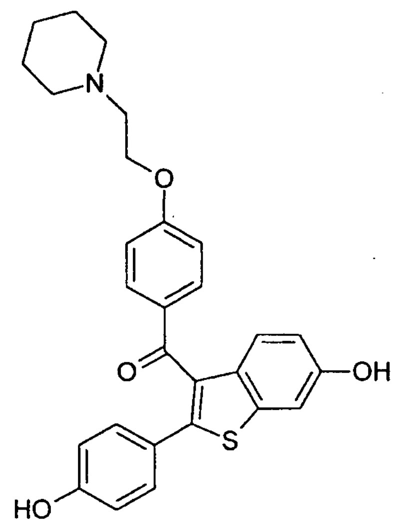


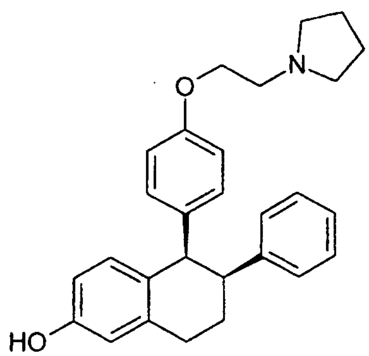


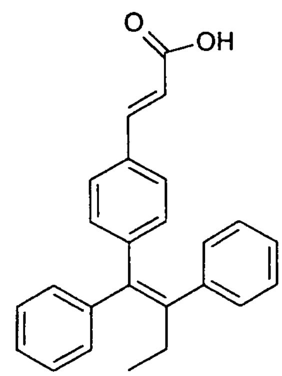
R^{12} = $-(CH_2)_{10}C(O)N(CH_3)CH_2CH_2CH_2CH_3$

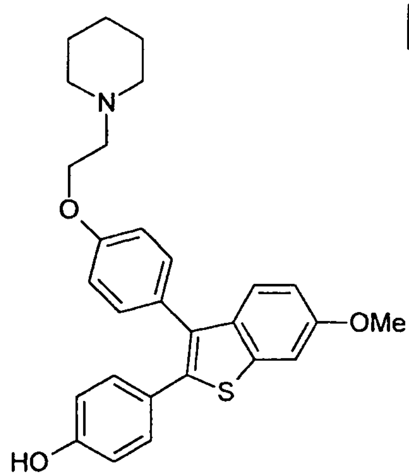
or $-(CH_2)_9S(O)(CH_2)_3CF_2CH_3$











wherein linkage to a linker occurs at any atom of the ligand capable of covalent attachment to the linker via conventional organic synthetic techniques as illustrated below.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20; and pharmaceutically acceptable salts thereof and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

Preferably, q is less than p.

In one of its method aspects, this invention is directed to a method for treating breast cancer mediated by estrogen receptors in a mammal which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound, or a pharmaceutically acceptable salt thereof, comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, at least two of said ligands comprising a ligand domain capable of binding to an estrogen receptor and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

In another of its method aspects, this invention is directed to a method for treating breast cancer, osteoporosis, or atherosclerosis mediated by estrogen

receptors in a mammal which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad \mathrm{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and pharmaceutically acceptable salts thereof and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

Preferably, q is less than p.

In another aspect, this invention is directed to general synthetic methods for generating large libraries or collections of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties against estrogen receptors. In one embodiment, the general synthetic methods employ combinatorial aspects to provide for libraries of multimeric compounds which compounds can then be assayed for multibinding properties against estrogen receptors. In another embodiment, a collection of multimeric compounds is prepared and processed through an iterative process to determine those molecular constraints necessary to impart multibinding properties against estrogen receptors.

In the library aspect, the diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers (i.e., a library of linkers) with a ligand or ligands (i.e., a library of ligands) to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of

linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity, polarization and polarizability. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

This invention is also directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties against estrogen receptors. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a ligand or a class of ligands targeting an estrogen receptor.

This invention is still further directed to iterative methods to determine those molecular constraints necessary to impart multibinding properties against estrogen receptors.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties against estrogen receptors which method comprises:

(a) identifying an estrogen receptor ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties against estrogen receptors.

In another of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties against estrogen receptors which method comprises:

(a) identifying a library of estrogen receptor ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties against estrogen receptors.

The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition of ligands is preferred when a mixture of different ligands is employed to ensure that heterodimeric or multimeric compounds are prepared. Concurrent addition of the ligands is preferred when it is desired that at least a portion of the to-be-prepared multimeric compounds will be homomultimeric compounds.

The assay protocols recited in (d) can be conducted on the multimeric ligand compound library produced in (c) above, or preferably, each member of the library can first be isolated, for example, by preparative liquid chromatography mass spectrometry (LCMS) and then assayed.

In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties against estrogen receptors which library is prepared by the method comprising:

(a) identifying an estrogen receptor ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties against estrogen receptors which library is prepared by the method comprising:

(a) identifying a library of estrogen receptor ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the

complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability, and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

In another preferred embodiment, the estrogen receptor ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, boronates, anhydrides, and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heteromeric (i.e., at least one of the ligands is different from the other ligands).

In addition to the combinatorial methods described herein, this invention provides for an interative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or

ligands targeting an estrogen receptor. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties against estrogen receptors which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target an estrogen receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties against estrogen receptor;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates preferred methods for linking amine containing ligands to provide for dimeric structures. In FIG. 1, R^2, Y and Z are as identified above and the figure illustrates how compounds of formula Ia and Ib could be prepared from conventional starting materials.

FIG. 2 illustrates conventional coupling chemistries for effecting coupling of a complementary reactive group on the linker with an amine group on the ligand wherein the shaded ball in the center of the coupling reagent is the linker entity.

FIG.s 3-18 illustrate specific reaction schemes for preparing dimeric multibinding compounds of this invention.

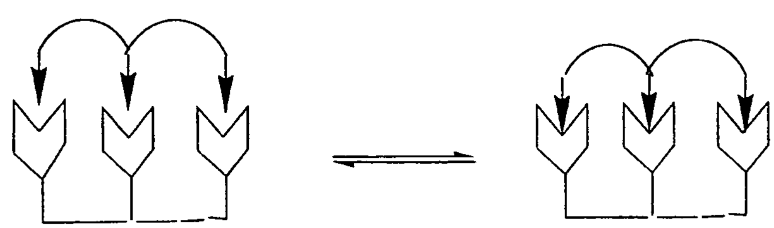
DETAILED DESCRIPTION OF THE INVENTION

As noted above, this invention is directed, in part, to multi-binding compounds that bind estrogen receptors.

The "affinity" and "specificity" of the estrogen receptor and a ligand thereto are dependent upon the complementarity of molecular binding surfaces and the energetic costs of complexation. "Affinity" is sometimes quantified by the equilibrium constant of complex formation. Specificity relates to the difference in affinity between the same ligand binding to different ligand binding sites on the cellular receptor.

The multi-binding compounds of this invention are capable of acting as multi-binding agents for estrogen receptors and the surprising activity of these compounds arises at least in part from their ability to bind in a multivalent manner with such mammalian estrogen receptors. Multivalent binding interactions are characterized by the concurrent interaction of multiple ligands with multiple ligand binding sites on one or more estrogen receptors. Multivalent interactions differ from collections of individual monovalent interactions by imparting enhanced biological and/or therapeutic effect.

Examples of multivalent binding interactions (e.g., trivalent) relative to monovalent binding interactions are shown below:



Just as multivalent binding can amplify binding affinities, it can also amplify differences in binding affinities, resulting in enhanced binding specificity as well as affinity.

Definitions:

Prior to discussing this invention in further detail, the following terms will first be defined.

The term "library" refers to at least 3, preferably from 10^2 to 10^9 and more preferably from 10^2 to 10^4 multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding properties against estrogen receptor. In another embodiment, each member of the library of multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties against estrogen receptor.

The term "collection" refers to a set of multimeric compounds which are prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to 10^9 members and still more preferably from 10 to 10^4 members.

The term "multimeric compound" refers to compounds comprising from 2 to 10 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms, and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl,
iso-butyl, *n*-hexyl, *n*-decyl, tetradecyl, and the like.

The term "substituted alkyl" refers to an alkyl group as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido,

cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene (-CH_2-), ethylene (-CH_2CH_2-), the propylene isomers (e.g., -$CH_2CH_2CH_2$- and -$CH(CH_3)CH_2$-) and the like.

The term "substituted alkylene" refers to an alkylene group, as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl,
-SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such fused groups contain from 1 to 3 fused ring structures.

The term "alkaryl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of example, methylenemethoxy ($-CH_2OCH_3$), ethylenemethoxy ($-CH_2CH_2OCH_3$), *n*-propylene-*iso*-propoxy ($-CH_2CH_2CH_2OCH(CH_3)_2$), methylene-*t*-butoxy ($-CH_2-O-C(CH_3)_3$) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are

alkylene-S-alkyl and include, by way of example, methylenethiomethoxy (-CH_2SCH_3), ethylenethiomethoxy (-$CH_2CH_2SCH_3$), *n*-propylene-*iso*-thiopropoxy (-$CH_2CH_2CH_2SCH(CH_3)_2$), methylene-*t*-thiobutoxy (-$CH_2SC(CH_3)_3$) and the like.

The term "alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. Preferred alkenyl groups include ethenyl (-$CH=CH_2$), *n*-propenyl (-$CH_2CH=CH_2$), *iso*-propenyl (-$C(CH_3)=CH_2$), and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "alkenylene" refers to a diradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. This term is exemplified by groups such as ethenylene (-$CH=CH$-), the propenylene isomers (e.g., -$CH_2CH=CH$- and -$C(CH_3)=CH$-) and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, and preferably from 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

The term "alkynyl" refers to a monoradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 20 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynyl groups include ethynyl ($-C\equiv CH$), propargyl ($-CH_2C\equiv CH$) and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic,

heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl,
-SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "alkynylene" refers to a diradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynylene groups include ethynylene (-C≡C-), propargylene ($-CH_2C{\equiv}C-$) and the like.

The term "substituted alkynylene" refers to an alkynylene group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl,
-SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "acyl" refers to the groups HC(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term “acylamino” or "aminocarbonyl" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term “aminoacyloxy” or "alkoxycarbonylamino" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl,

substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl,
-SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to the diradical derived from aryl (including substituted aryl) as defined above and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group $-NH_2$.

The term "substituted amino refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" or "alkoxycarbonyl" refers to the groups "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl",

"-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "cycloalkylene" refers to the diradical derived from cycloalkyl as defined above and is exemplified by 1,1-cyclopropylene, 1,2-cyclobutylene, 1,4-cyclohexylene and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "substituted cycloalkylene" refers to the diradical derived from substituted cycloalkyl as defined above.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring and at least one point of internal

unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term “cycloalkenylene” refers to the diradical derived from cycloalkenyl as defined above and is exemplified by 1,2-cyclobut-1-enylene, 1,4-cyclohex-2-enylene and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term “substituted cycloalkenylene” refers to the diradical derived from substituted cycloalkenyl as defined above.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

The term “pseudohalide” refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups include, by way of example, mesyl, tosyl, azido and cyano groups.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl,
-SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

The term “heteroaryloxy” refers to the group heteroaryl-O-.

The term “heteroarylene” refers to the diradical group derived from heteroaryl (including substituted heteroaryl), as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylene, 1,8-quinolinylene, 1,4-benzofuranylene, 2,5-pyridnylene, 2,5-indolenyl and the like.

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl,
-SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term “heterocyclene” refers to the diradical group formed from a heterocycle, as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term “oxyacylamino” or "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term “spiro-attached cycloalkyl group” refers to a cycloalkyl group attached to another ring via one carbon atom common to both rings.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds (including intermediates thereof) prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable thiol blocking groups include disulfide groups, acyl groups, benzyl groups, and the like.

Preferred removable amino blocking groups include conventional substituents such as t-butyoxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (FMOC), allyloxycarbonyl (ALOC), and the like which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, *t*-butyl etc. which can be removed by mild conditions compatible with the nature of the product.

The term "pharmaceutically-acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid

and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically-acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

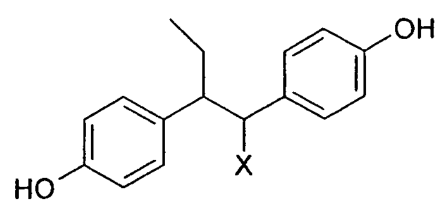
Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should

also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "pharmaceutically-acceptable cation" refers to the cation of a pharmaceutically-acceptable salt.

The term "hexestrol" refers to compounds of the formula:



where X is selected from the group consisting of a carboxyl group, a carboxyl ester, and a thioether as set forth in Bergmann, et al., *J. Steriod Biochem. Molec. Biol.*, 49(2/3):139-152 (1994). Dimers disclosed by Bergmann, et al. are specifically excluded from the claims of this invention.

The term "modulate" refers to a change that is effected when a ligand binds to an estrogen receptor. The multibinding compounds of this invention

may modulate the estrogen receptor as either an agonist, partial agonist, antagonist or inverse antagonist as described above. A ligand which has dual agonist/antagonist properties is known as a selective estrogen receptor modulator ("SERM"). In the case of a SERM, the modulating effect of a ligand depends upon the cell or tissue type in which the ER is found.

The estrogen receptor ("ER") is a member of the nuclear receptor superfamily of transcription factors. In the absence of hormone it resides in the nucleus of target cells in a transcriptionally inactive state. Upon binding ligand, ER undergoes a conformational change, initiating a cascade of events leading ultimately to its association with specific regulatory regions within target genes.[10] The ensuing effect on transcription is influenced by the cell and promoter context of the DNA-bound receptor. It is in this manner that the physiological ER agonist, estradiol, exerts its biological activity in the reproductive, skeletal and cardiovascular systems.

Further, the binding of estrogen to the ER stimulates the increased expression of some genes, including those for some growth factors and growth factor receptors resulting in the stimulation of DNA synthesis and cell proliferation, as well as the increased production of proteins such as plasminogen activator and collagenases that are believed to enhance the metastatic capability of breast cancer cells.[7] When an ER antagonist binds to the ER, the receptor is not available to bind estrogen. Consequently, the antagonist-ER complex fails to effectively stimulate gene expression and DNA synthesis; instead, the antagonist-ER complex enhances production of some growth inhibitory factors, including the TGF-βs, thereby preventing breast cancer growth and metastasis.[7]

In addition to these activities, estrogen has been shown to function as a mitogen in most ER-positive breast cancer cells. Thus, treatment regimens that include antiestrogens (antagonists), compounds that oppose the actions of estrogen, have been effective clinically in halting or delaying the progression of

the disease.[8] One compound which has been extensively studied for its function as an antagonist in most ER-positive breast cancer tumors is tamoxifen.[8] Tamoxifen is also known to show agonist activity in bone and the cardiovascular system and shows partial agonist activity in the uterus.[8] Thus, the agonist/antagonist activity of the ER-tamoxifen complex is influenced by cell context.[8]

Accordingly, the multibinding compounds of this invention may exhibit agonist and/or antagonist activities, depending on the cell type that is targeted. For example, the multibinding compounds may be used to treat breast cancer, osteoporosis, atherosclerosis and other disease states in which the ER is implicated.

It should be recognized that the estrogen receptors that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations (e.g. cellular receptors may be covalently joined in a single structure, noncovalently associated in a multimeric structure, embedded in a membrane or polymeric matrix and so on) and therefore have less translational and rotational freedom than if the same cellular receptors were present as monomers in solution.

The term "ligand binding site" as used herein denotes the site on the estrogen receptor that recognizes a ligand domain and provides a binding partner for that ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example agonism, antagonism, modulatory effect and the like or may maintain an ongoing biological event.

"Ligand" or "estrogen receptor ligand" as used herein denotes a compound that is a binding partner for the estrogen receptor and is bound thereto by complementarity. The specific region or regions of the ligand that is (are)

recognized by the estrogen receptor is designated as the "ligand domain". A ligand may be either capable of binding to a receptor by itself, or may require the presence of one or more non-ligand components for binding (e.g., Ca^{+2}, Mg^{+2} or a water molecule).

It is further understood that the term "ligand" or "estrogen receptor ligand" is not intended to be limited to compounds known to be useful as estrogen receptor binding compounds (e.g., known drugs). However, it should be understood that portions of the ligand structure that are not essential for specific molecular recognition and binding activity may be varied substantially, replaced with unrelated structures and, in some cases, omitted entirely without affecting the binding interaction. The primary requirement for the ligand is that it has a ligand domain as defined above. Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not normally associated with estrogen cellular receptor binding properties. In addition, it should be noted that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multivalent compounds because of the benefits conferred by multi-valency. The only requirement for a ligand is that it has a ligand binding domain as defined above. The ligands and linkers which comprise the multibinding agents of the invention and the multibinding compounds themselves may have various stereoisomeric forms, including enantiomers and diastereomers. It is to be understood that the invention contemplates all possible stereoisomeric forms of multibinding compounds, and mixtures thereof.

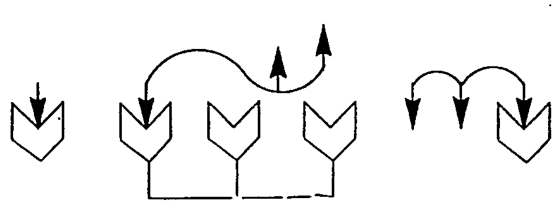
Accordingly, examples of ligands useful for this invention include tamoxifen, toremifene, idoxifene, droloxifene, miproxifene, levomeloxifene, raloxifene, and Fulvestrant.

A "multi-binding agent" or "multi-binding compound" refers to a compound that is capable of multivalency as defined below, and which has 2 to

10 ligands covalently bound to one or more linkers which may be the same or different wherein at least 1 of the ligands comprise a ligand domain capable of binding to one or more estrogen receptors. The multi-binding compound provides a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which may be the same or different which unlinked ligands comprise a ligand domain capable of binding to one or more estrogen receptors. That is to say that the biological and/or therapeutic effect of the estrogen receptor binding ligands attached to the multi-binding compound is greater than that achieved by the same amount of unlinked estrogen receptor ligands made available for binding to the ligand binding sites.

The phrase "increased biological or therapeutic effect" includes, for example increased affinity for a target, increased specificity for a target, increased selectivity for a target, increased potency, increased efficacy, decreased toxicity, improved duration of action, decreased side effects, increased therapeutic index, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multi-binding compounds of this invention will exhibit at least one and preferably more than one of the above mentioned effects.

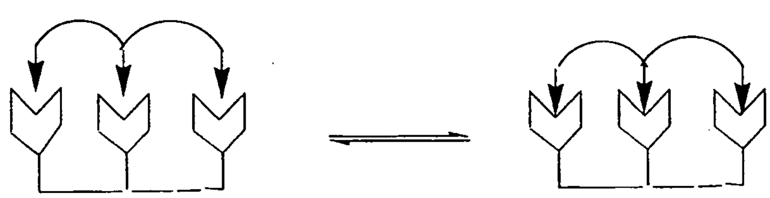
"Uni-valency" as used herein refers to a single binding interaction between one ligand as defined herein with one ligand binding site as defined herein. It should be noted that a molecule having multiple copies of a ligand (or ligands) exhibits uni-valency when only one ligand is interacting with a ligand binding site. Examples of a univalent interaction are depicted below.



"Multi-valency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands (which may be the same or different) and two or more corresponding ligand binding sites on the receptors which receptors may be the same or different.

For example, two ligands connected by a linker that bind concurrently to two ligand binding sites would be considered as bi-valency; three ligands thus connected would be an example of tri-valency. An example of tri-valency illustrating a multi-binding agent bearing three ligands versus a univalent binding interaction is shown below:

univalent interaction



trivalent interaction

It should be understood that all compounds that contain multiple copies of a ligand attached to a linker do not necessarily exhibit the phenomena of multi-valency, i.e., that the biological and/or therapeutic effect of the multi-binding agent is greater than the sum of the aggregate of unlinked ligands made available to the ligand binding site. For multivalency to occur, the ligands that are connected by a linker have to be presented to their receptors by the linker in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multi-binding agent.

"Potency" as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multi-binding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g. an *in vitro* or *in vivo* assay, in an appropriate animal model such as a human patient). The finding that the multi-binding agent produces an equivalent biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g. on a per weight, per mole or per ligand basis) is indicative of enhanced potency.

"Selectivity" or "specificity" is a measure of the binding preferences of a ligand for different ligand binding sites. The selectivity of a ligand with respect to its target ligand binding site relative to another ligand binding site is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex) or in cases where a biological effect is observed below the K_d, the ratio of the respective EC_{50} s (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct ligand binding sites).

The terms "agonism" and "antagonism" are well known in the art. The term "modulatory effect" refers to the ability of the ligand to change the activity of an agonist or antagonist through binding to a ligand binding site.

The term "partial agonist" refers to a receptor agonist which cannot fully elicit a maximal response when it binds to the receptor, no matter how high the concentration of the partial agonist. A partial agonist is able to combine with the receptor, but the full effect of the binding is not elicited. This term is well known in the art and a discussion of it may be found in Textbook of Receptor Pharmacology, ch 1.4, J. Foreman and T. Johansen eds., CRC Press, 1996.

The term "treatment" refers to the treatment of estrogen receptor-associated disease, in particular breast cancer and osteoporosis, and includes:

(i) preventing disease from occurring in a subject and, accordingly, the treatment constitutes prophylactic treatment;

(ii) inhibiting disease, i.e., arresting the occurrence of disease; or

(iii) relieving or reducing the duration, intensity and/or severity of disease.

The term "therapeutically effective amount" refers to that amount of multi-binding compound which is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "linker," identified where appropriate by the symbol “X”, refers to a group or groups that covalently link(s) from 2 to 10 ligands (as identified above) in a manner that provides for a compound capable of multi-valency when in the presence of at least one cellular receptor having 2 or more ligand binding sites. The linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. In some cases the linker may be biologically active. The term linker does not, however, extend to cover solid inert supports such as beads, glass particles, fibers and the like. But it is to be understood that the multi-binding compounds of this invention can be attached to a solid support if desired, for example, for use in separation and purification processes and for similar applications.

The extent to which multivalent binding is realized depends upon the efficiency with which the linker or linkers that joins the ligands presents them to

their ligand binding sites on one or more receptors. Beyond presenting ligands for multivalent interactions with ligand binding sites, the linker spatially constrains these interactions to occur within dimensions defined by the linker. Thus the structural features of the linker (valency, geometry, orientation, size, flexibility, chemical composition) are features of multivalent compounds that play an important role in determining their activities.

The linkers used in this invention are selected to allow multivalent binding of ligands to any desired ligand binding sites of a receptor, whether such sites are located interiorly, both interiorly and on the periphery of the molecule, or at any intermediate position thereof. In one embodiment, the distance between the nearest neighboring ligand domains is preferably in the range of about 2Å to about 100Å, more preferably in the range of about 3Å to about 40Å.

The ligands are covalently attached to the linker or linkers using conventional chemical techniques providing for covalent linkage of the ligand to the linker or linkers. The reaction chemistry resulting in such linkage are well known in the art and involve the use of complementary functional groups on the linker and ligand. Preferably, the complementary functional groups on the linker are selected relative to the functional groups available on the ligand for binding or which can be introduced onto the ligand for binding. Again, such complementary functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker.

There are numerous complementary reactive groups and the resulting bonds formed by reaction there between. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March[1].

The linker is attached to the ligand at a position that retains ligand binding domain-receptor binding and specifically which permits the receptor recognition site of the ligand to orient itself to bind to the receptor. Such positions and synthetic protocols for linkage are well known in the art. Following attachment to the linker or a significant portion thereof (e.g. 2-10 atoms of linker), the linker-ligand conjugate is tested for retention of activity in a relevant assay system. If a linker-ligand conjugate shows activity at a concentration of less than 1 mM, it is considered to be acceptable for use in constructing a multi-binding compound. The relative orientation in which the ligand domains are displayed to the receptors depends both on the particular point (or points) of attachment of the ligands to the linker, and on the framework geometry. The term linker embraces everything that is not considered to be part of the ligand.

Suitable linkers are discussed below.

At present, it is preferred that the multi-binding agent is a bivalent compound, e.g., two ligands which are covalently linked to linker X.

<u>Methodology</u>

The linker, when covalently attached to multiple copies of the ligand, provides a biocompatible, substantially non-immunogenic multi-binding compound of this invention. The biological activity of the multi-binding compound is highly sensitive to the valency, geometry, composition, size, flexibility or rigidity, etc. of the linker as well as the presence or absence of anionic or cationic charge, the relative hydrophobicity/hydrophilicity of the

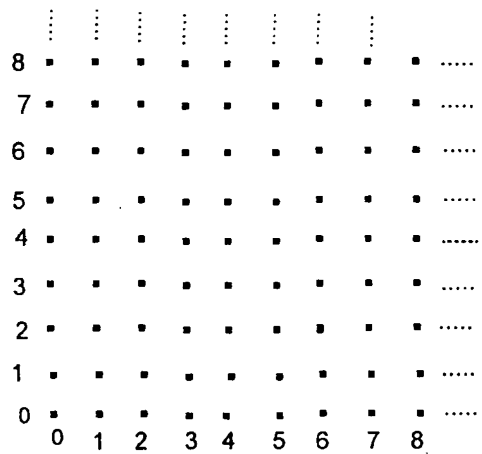
linker, and the like on the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the multi-binding compound. The linker may be biologically "neutral", i.e., not itself contribute any biological activity to the multi-binding compound or it may be chosen to enhance the biological activity of the molecule. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands to the receptors to permit multi-valency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multi-binding compound.

For example, different orientations can be achieved by including in the framework groups mono- or polycyclic groups, aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). Other groups can also include oligomers and polymers which are branched- or straight-chain species. In one preferred embodiment, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.) In another preferred embodiment, the ring is a six or ten member ring. In still further preferred embodiments, the ring is an aromatic ring such as, for example, phenyl or naphthyl.

The presentation of different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moieties can readily be achieved by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ($H_2N(CH_2)_6NH_2$) or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines".

Different frameworks can be designed to provide preferred orientations of the ligands. Such frameworks may be represented by using an array of dots (as

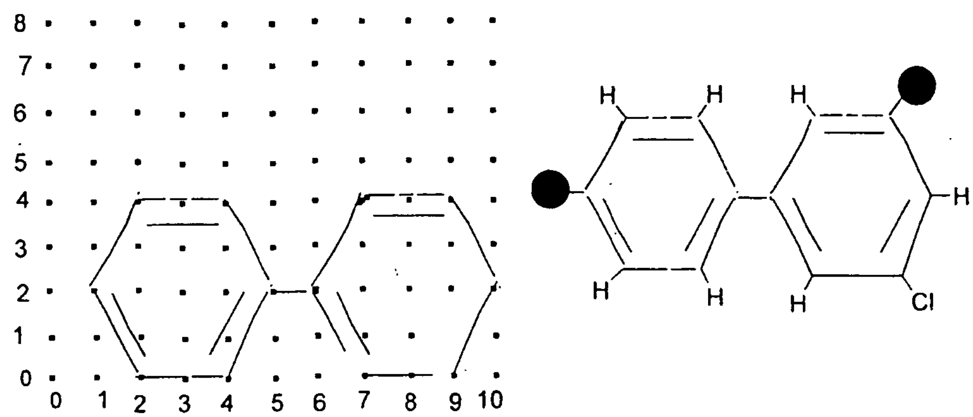
shown below) wherein each dot may potentially be an atom, such as C, O, N, S, P, H, F, Cl, Br, and F or the dot may alternatively indicate the absence of an atom at that position. To facilitate the understanding of the framework structure, the framework is illustrated as a two dimensional array in the following diagram, although clearly the framework is a three dimensional array in practice:



Each dot is either an atom, chosen from carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, or halogen, or the dot represents a point in space (i.e., an absence of an atom). Only certain atoms on the grid have the ability to act as an attachment point for the ligands, namely, C, O, N, S and P.

Atoms can be connected to each other via bonds (single, double or triple bonds with acceptable resonance and tautomeric forms), with regard to the usual constraints of chemical bonding. Ligands may be attached to the framework via single, double or triple bonds (with chemically acceptable tautomeric and resonance forms). Multiple ligand groups (2 to 10) can be attached to the framework such that the minimal, shortest path distance between adjacent ligand groups does not exceed 100 atoms. Preferably, the linker connections to the ligand is selected such that the maximum spatial distance between two adjacent ligands is no more than 40 Angstroms (Å).

An example of a linker as presented by the grid is shown below for a biphenyl construct.



Nodes (1,2), (2,0), (4,4), (5,2), (4,0), (6,2), (7,4), (9,4), (10,2), (9,0), (7,0) all represent carbon atoms. Node (10,0) represents a chlorine atom). All other nodes (or dots) are points in space (i.e., represent an absence of atoms. Nodes (1,2) and (9,4) are attachment points. Hydrogen atoms are affixed to nodes (2,4), (4,4), (4,0), (2,0), (7,4), (10,2) and (7,0). Nodes (5,2) and (6,2) are connected by a single bond.

The carbon atoms present are connected by either a single or double bonds, taking into consideration the principle of resonance and/or tautomerism.

The intersection of the framework (linker) and the ligand group, and indeed, the framework (linker) itself can have many different bonding patterns. Examples of acceptable patterns of three contiguous atom arrangements are shown in the following diagram:

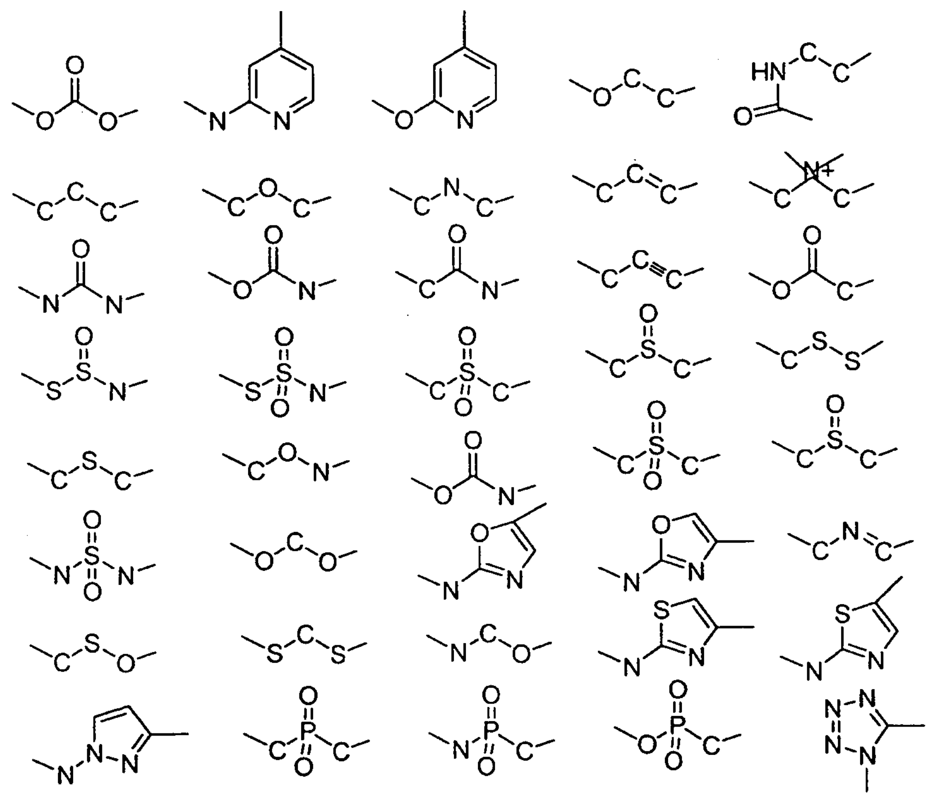
CCC	NCC	OCC	SCC	PCC
CCN	NCN	OCN	SCN	PCN
CCO	NCO	OCO	SCO	PCO
CCS	NCS	OCS	SCS	PCS
CCP	NCP	OCP	SCP	PCP
CNC	NNC	ONC	SNC	PNC
CNN	NNN	ONN	SNN	PNN
CNO	NNO	ONO	SNO	PNO
CNS	NNS	ONS	SNS	PNS
CNP	NNP	ONP	SNP	PNP
COC	NOC	OOC	SOC	POC
CON	NON	OON	SON	PON
COO	NOO	OOO	SOO	POO
COS	NOS	OOS	SOS	POS
COP	NOP	OOP	SOP	POP
CSC	NSC	OSC	SSC	PSC
CSN	NSN	OSN	SSN	PSN
CSO	NSO	OSO	SSO	PSO
CSS	NSS	OSS	SSS	PSS
CSP	NSP	OSP	SSP	PSP
CPC	NPC	OPC	SPC	PPC
CPN	NPN	OPN	SPN	PPN
CPO	NPO	OPO	SPO	PPO
CPS	NPS	OPS	SPS	PPS
CPP	NPP	OPP	SPP	PPP

One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in March[1]. These arrangements are described in the grid of dots shown in the scheme above. All of the possible arrangements for the five most preferred atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

The identification of an appropriate framework geometry for ligand domain presentation is an important first step in the construction of a multivalent

binding agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process.

Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.



It can therefore be seen that there is a plethora of possibilities for the composition of a linker. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatic groups, ethers, lipids, cationic or anionic groups, or a combination thereof. A wide diversity of linkers is commercially available from Chemsources USA; ChemSources International and ACD. Many of the linkers that are suitable for use in this invention fall into this

category. Others can be readily synthesized by methods known in the art and described below.

Assay of each of the individual compounds of a collection generated as described above will lead to a subset of compounds with the desired enhanced activities (e.g. potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will provide a framework orientation that favors the properties desired. Having selected a preferred framework geometry, the physical properties of the linker can be optimized by varying the chemical composition. The composition of a linker can be varied in numerous ways to achieve the desired physical properties.

Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multi-binding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto the linker enhances the hydrophilicity and water solubility of the multi-binding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further PEG decreases antigenicity and potentially enhances the overall rigidity of the linker.

Ancillary groups which enhance the water solubility/hydrophilicity of the linker and, accordingly, the resulting multi-binding compounds are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, poly(ethylene glycols), alcohols, polyols, (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligo- and polysaccharides, etc.) carboxylates, polycarboxylates, (e.g.,

polyglutamic acid, polyacrylic acid, etc.), amines, polyamines, (e.g., polylycine, poly(ethyleneimine), and the like) to enhance the water solubility and/or hydrophilicity of the multi-binding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether. In particularly preferred embodiments, the ancillary group will be a poly(ethylene glycol).

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the multi-binding compounds described herein is within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, by way of example only, aryl and heteroaryl groups which, as above, may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives which do not form bilayers in aqueous medium until higher concentrations are reached.

Also within the scope of this invention is the use of ancillary groups which result in the multi-binding compound being incorporated into a vesicle such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic

acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker or bonds between the linker and the ancillary group(s) or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the presence of rings and/or multiple bonds, for example, aryl, heteroaryl, cycloalkyl and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker into a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge when deprotected, following the addition to the linker, include deprotectation of a carboxyl, hydroxyl, thiol or

amino protecting group, by a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl. In other preferred embodiments, the linker comprises one or more six-membered rings or crown groups which, while not rigid, retain the conformation of the linker through conformational entropy.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art. Eliminating or reducing antigenicity of the multi-binding compounds described herein is also within the scope of this invention.

As explained above, the multi-binding compounds described herein comprise 2-10 ligands for estrogen attached to a linker that links the ligands in such a manner that they are presented to the estrogen receptor for multivalent interactions. The linker spatially constrains these interactions to occur within dimensions defined by the linker, thus greatly increasing biological activity of

the multi-binding compound as compared to the same number of ligands used in mono-binding form.

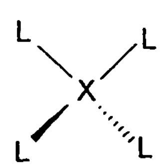
The multi-binding compounds of this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multi-valency, and a more detailed explanation is described below.

As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position.

The simplest and most preferred multi-binding compound is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different and X is the linker. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as can X. However, a trimer can also be a multi-binding compound comprising three ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group. Tetravalent compounds can be represented as, for example, in a linear array:

L-X-L-X-L-X-L

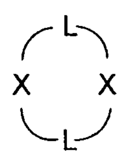
or in a tetrahedral array:



where X and L are as defined herein.

The same considerations apply to higher multibinding compounds of this invention containing 5-10 ligands. However, for multibinding agents attached to a central linker such as aryl or cycloalkyl, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not directly accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

Certain of the above described compounds may alternatively be represented as cyclic chains of the form:



and variants thereof.

All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$.

In view of the above description of the linker, it is understood that the term "linker" when used in combination with the term "multibinding compound" includes both a covalently contiguous single linker (e.g., L-X-L) and multiple covalently non-contiguous linkers (L-X-L-X-L) within the multibinding compound.

Preferred multibinding compounds of this invention are dimeric including both homodimeric and heterodimeric structures.

Preparation of Multibinding Compounds

The multibinding compounds of this invention can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

Any compound which inhibits estrogen can be used as a ligand in this invention. As discussed in further detail below, numerous such estrogen inhibitors are known in the art and any of these known compounds or derivatives thereof may be employed as ligands in this invention.

The multibinding compounds of this invention are preferably prepared by reacting at least two stoichiometric equivalents of a ligand or a mixture of ligands having a first functional group with a linker or mixture of linkers having at least two functional groups complementary to the functional groups on the ligand such that the complementary functional groups can react under suitable conditions to

form a covalent bond between the ligands and the linker. Preferred multibinding compounds are dimeric compounds and the discussion below is directed to such dimeric structures. It is understood, of course, that the discussion below can be readily extended to other multimeric structures including trimers, tetramers, etc.

FIG. 1 illustrates this concept in general terms wherein diamine reagent **5** or **5'** (the shaded ball representing the remainder of the linker) is reacted, in a first instance, with a complementary functional group X (e.g., a halo group) on an estrogen receptor ligand **2** to provide for a dimer of formula Ia. In a second instance, diamine **5'** is reacted with a carboxylic acid functional group of an estrogen receptor ligand **4** to provide for dimer **Ib** (where Q is an alkylene chain). The inverse of these reactions is illustrated in the second reaction scheme of FIG. 1 wherein a primary or secondary amine functional group of an estrogen receptor ligand **3** is reacted with a complementary functional group of difunctionalized linker **5"** wherein W is the complementary functional group.

Specific illustrations of these reactions are depicted in FIG. 2 wherein difunctionalized linker **5"** is represented by dicarboxylic acid **10**, dicarboxylic acid halide **11**, dialdehyde **12**, dihalo **13** (X = is a halo group. It can also be other leaving groups such as mesylate, tosylate, etc.), diepoxide **14**, disulfonylhalide **15** and diisocyanate **16**. Each of these functional groups is complementary to the amine functional group of estrogen receptor ligand **3** by virtue of the fact that this amine group will react with each of these groups to provide for a covalent linkage. As illustrated in FIG. 2, both dicarboxylic acid **10** and dicarboxylic acid halide **11** react with the amine functional group of estrogen receptor ligand **3** under conventional conditions to provide for an amide linkage between the ligand and the linker.

Each of such reactions are conventional and well known in the art. For example, amide formation merely involves coupling of a suitable acid derivative of dicarboxylic acid **10** with the amine of estrogen receptor ligand **3** under

conditions which provide for the amide linked dimer. This reaction is conventionally conducted for peptide synthesis and synthetic methods used therein can also be employed to prepare these dimers of this invention. For example, well known coupling reagents such as carbodiimides with or without the use of well known additives such as N-hydroxysuccinimide, 1-hydroxybenzotriazole, etc. can be used to facilitate coupling. The reaction is conventionally conducted in an inert aprotic diluent such as dimethylformamide, dichloromethane, chloroform, acetonitrile, tetrahydrofuran and the like.

Alternatively, the dicarboxylic acid halide **11** can be employed in this reaction and, when so employed, it is typically employed in the presence of a suitable base to scavenge the acid generated during the reaction. Suitable bases include, by way of example, triethylamine, diisopropylethylamine, N-methylmorpholine and the like.

Reaction of dialdehyde **12** with the amine of estrogen receptor ligand **3** is conducted under conventional reductive amination conditions wherein a first aldehyde group of dialdehyde **12** reacts with the amine to form an imine group (not shown) which is reduced *in situ* in the presence of a reducing agent such as sodium cyanoborohydride to generate an amine linkage between the ligand and the linker.

Further conventional reactions to generate an amine linkage between the ligand and the linker are illustrated by reaction of dihalo linkers **13** with the amine group of estrogen receptor ligand **3** and by epoxide ring opening reactions between a diepoxide linker **14** and the amine group of estrogen receptor ligand **3**.

Sulfonylhalides **15** are well known in the art as well as their reaction with amines to provide for sulfonamide linkages. Such reactions are typically conducted in an inert diluent in the presence of an excess of base which is

employed to scavenge the acid generated by the reaction. Suitable bases include, by way of example, pyridine, triethylamine, diisopropylethylamine, and the like.

Similarly, ureas are conventionally formed by reaction of an isocyanate **16** with an amine group of estrogen receptor ligand **3**.

Specific illustrations of some of these reactions are depicted in FIGs. 3-13.

FIG. 3 illustrates reaction between estrogen receptor ligand **2a** having a halo (chloro) functional group and triamine **5a**. The reaction employs at least 2 equivalents of ligand **2a** relative to triamine **5a** and is conducted in an inert diluent preferably in the presence of a base to scavenge the acid generated by the reaction. The reaction is conventional and provides for dimer in high yield which can be purified by conventional techniques including chromatography, filtration and the like.

FIG. 3 also illustrates a reaction scheme for preparing estrogen receptor ligand **2a** from known starting material **21** (Miller, et al., J. Org. Chem., 50:2121-2123 (1985). Specifically, FIG. 3 illustrates reaction of at least a stoichiometric amount of compound **21** with 2-chloroethanol **22** in an inert diluent such as tetrahydrofuran (THF) in the presence of triphenylphosphine (Ph_3P) and diethyl azodicarboxylate (DEAD). The reaction is preferably conducted at room temperature for approximately 5 hours to provide for compound **21**.

FIG. 4 illustrates reaction between estrogen receptor ligand **2b** having a halo (bromo) functional group and diamine **5b**. The reaction employs at least 2 equivalents of ligand **2b** relative to diamine **5b** and is conducted in an inert diluent preferably in the presence of a base to scavenge the acid generated by the reaction. The reaction is conventional and provides for dimer in high yield

which can be purified by conventional techniques including chromatography, filtration and the like.

FIG. 4 also illustrates a reaction scheme for preparing estrogen receptor ligand **2b** from known starting material **24** (Tatee, et al., J. Med. Chem., 50:1509-1517 (1979). Specifically, FIG. 4 illustrates reaction of at least a stoichiometric amount of compound **24** with 1,2-dibromoethane **25** in an inert diluent such as acetone in the presence of base (K_2CO_3) to scavenge the acid generated. The reaction is preferably conducted at 50°C for approximately 15 hours to provide for compound **26**. Demethylation of compound **26** by conventional conditions (boron tribromide in dichloromethane) provides for compound **2b**.

FIG. 5 illustrates reaction between estrogen receptor ligand **2c** having a halo (bromo) functional group and diamine **5c**. The reaction employs at least 2 equivalents of ligand **2c** relative to diamine **5c** and is conducted in an inert diluent preferably in the presence of a base to scavenge the acid generated by the reaction. The reaction is conventional and provides for dimer in high yield which can be purified by conventional techniques including chromatography, filtration and the like.

FIG. 5 also illustrates a reaction scheme for preparing estrogen receptor ligand **2c** from known starting material **27** (Tatee, et al., J. Med. Chem., 50:1509-1517 (1979)). Specifically, FIG. 5 illustrates reaction of at least a stoichiometric amount of compound **27** with 1;2-dibromoethane **25** in an inert diluent such as acetone in the presence of base (K_2CO_3) to scavenge the acid generated. The reaction is preferably conducted at 50°C for approximately 15 hours to provide for compound **28**. Demethylation of compound **28** by conventional conditions (boron tribromide in dichloromethane) provides for compound **2c**.

FIG. 6 illustrates reaction between estrogen receptor ligand **2d** having a halo (bromo) functional group and diamine **5d**. The reaction employs at least 2 equivalents of ligand **2d** relative to diamine **5d** and is conducted in an inert diluent preferably in the presence of a base to scavenge the acid generated by the reaction. The reaction is conventional and provides for dimer in high yield which can be purified by conventional techniques including chromatography, filtration and the like.

FIG. 6 also illustrates a reaction scheme for preparing estrogen receptor ligand **2d** from known starting material **29** (Crenshaw, et al., J. Med. Chem., 14:1185-1190 (1971)). Specifically, FIG. 6 illustrates reaction of compound **29** with approximately 0.5 equivalents of 1,2-dibromoethane **25** in an inert diluent such as acetone in the presence of base (K_2CO_3) to scavenge the acid generated. The reaction is preferably conducted at 50°C for approximately 15 hours to provide for compound **2d** which can be purified by conventional methods such as chromatography.

FIG. 7 illustrates reaction between estrogen receptor ligand **2e** following the procedures of Jones et al., *J. Med. Chem.*, 27:1057-1066 (1984), having a halo (chloro) functional group and diamine **5e**. The reaction employs at least 2 equivalents of ligand **2e** relative to diamine **5e** and is conducted in an inert diluent preferably in the presence of a base to scavenge the acid generated by the reaction. The reaction is conventional and provides for dimer in high yield which can be purified by conventional techniques including chromatography, filtration and the like.

FIG. 7 also illustrates a reaction scheme for preparing diamine **5e** from known starting material **30** (Aldrich, Registry #64028-78-0). Specifically, FIG. 7 illustrates blocking of the amine groups of compound **30** with Boc (t-butoxycarbonyl) provides for a di-Boc compound (not shown) which can be

reduced with lithium aluminum hydride (LAH) to provide for compound **5e** which can be purified by conventional methods such as chromatography.

FIG. 8 illustrates reaction between estrogen receptor ligand **3a** having an amino (methylamino) functional group and dicarboxylic acid **10a**. The reaction employs at least 2 equivalents of ligand **3a** relative to dicarboxylic acid **10a** and can optionally employ well known coupling reagents such as carbodiimides with or without the use of well known additives such as N-hydroxysuccinimide, 1-hydroxybenzotriazole, etc. The reaction is conventionally conducted in an inert aprotic diluent such as dimethylformamide, dichloromethane, chloroform, acetonitrile, tetrahydrofuran and the like. The resulting product can be purified by conventional methods such as chromatography.

FIG. 8 also illustrates a reaction scheme for preparing estrogen receptor ligand **2a** (described above). Specifically, FIG. 8 illustrates reaction of at least a stoichiometric amount of compound **2a** with methylamine, typically in excess, in an inert diluent such as methanol. The reaction is preferably conducted at room temperature to provide for compound **3a** which can be purified by conventional methods such as chromatography.

FIG. 9 illustrates reaction between estrogen receptor ligand **3b** having a primary amino functional group and dialdehyde **12a**. The reaction employs at least 2 equivalents of ligand **3b** relative to dialdehyde **12a** to form diimine intermediate (not shown) which can be reduced by conventional reducing agents (sodium cyanoborohydride in methanol) to provide for the diamine dimer which can be purified by conventional methods such as chromatography.

FIG. 9 also illustrates a reaction scheme for preparing estrogen receptor ligand **3b** from ligand **2b** (described above). Specifically, FIG. 9 illustrates reaction of compound **2b** with ammonia, typically in excess, in an inert diluent such as tetrahydrofuran (THF). The reaction is preferably conducted at elevated

temperatures (e.g., 60°C) for approximately 12 hours and preferably in a sealed vessel or tube to provide for compound **3b** which can be purified by conventional methods such as chromatography.

In addition, FIG. 9 illustrates conversion of an ethylene glycol **31** to the corresponding α,ω-dialdehyde **12a** by conventional oxidation conditions using PCC (pyridinium chlorochromate) as described by March, J., Advanced Organic Chemistry 4th Edition, p. 1168-1169 (1992). The resulting product can be purified by conventional methods such as chromatography.

FIG. 10 illustrates reaction between estrogen receptor ligand **3c** having an amino functional group and dihalide **13a**. The reaction employs at least 2 equivalents of ligand **3c** relative to dihalide **13a** to form the diamine dimer which can be purified by conventional methods such as chromatography. The reaction is conventional and is described in the art. The resulting product can be purified by conventional methods such as chromatography.

FIG. 10 also illustrates a reaction scheme for preparing estrogen receptor ligand **3c** from ligand **2c** (described above). Specifically, FIG. 10 illustrates reaction of compound **2b** with methylamine, typically in excess, in an inert diluent to form compound **3c** which can be purified by conventional methods such as chromatography.

In addition, FIG. 10 illustrates conversion of an ethylene glycol **32** to the corresponding α,ω-dihalide **13a** again by use of conventional conditions using, for example, phosphorus tribromide as described, for example, by March, J., Advanced Organic Chemistry, 4th Edition, pp. 431 (1992).

FIG. 11 illustrates reaction between estrogen receptor ligand **3d** having a secondary amino functional group and dibromide **13b**. The reaction employs at least 2 equivalents of ligand **3d** relative to dibromide **13b** to form the diamine

linked dimer which can be purified by conventional methods such as chromatography.

FIG. 11 also illustrates a reaction scheme for preparing estrogen receptor ligand **3d** from ligand **2d** (described above). Specifically, FIG. 11 illustrates reaction of compound **2d** with ethylamine, typically in excess, in an inert diluent such as tetrahydrofuran (THF) to provide for compound **3d** which can be purified by conventional methods such as chromatography.

Reactions similar to those described above are illustrated in FIG. 12 and 13 which depict conventional amide formation to form dimeric multibinding compounds of this invention. In both cases, amide formation can optionally employ well known coupling reagents such as carbodiimides with or without the use of well known additives such as N-hydroxysuccinimide, 1-hydroxybenzotriazole, etc. The reaction is conventionally conducted in an inert aprotic diluent such as dimethylformamide, dichloromethane, chloroform, acetonitrile, tetrahydrofuran and the like. The resulting product can be purified by conventional methods such as chromatography.

The methods of this invention are further exemplified in reaction schemes 1 through 5 illustrated in Figures 14-18 respectively. Specifically, reaction scheme 1 first illustrates conventional amide formation to form a dimeric multibinding compound of this invention from steroidal estrogen receptor ligand **40** which is disclosed by Bowler, et al., Steroids, 1989, 71. At least two equivalents of ligand **40** is combined with diamine linker (the linker is shown as a box), DCC (dicyclohexyldicarbiimide), DMAP (dimethylaminopyridine), HOBt (1-hyrdoxybenzotriazole) and DMF (dimethylformamide) under conditions to form dimer **41**.

Reaction scheme 1 also illustrates an alternative synthesis of compound **3a** by combining at least stoichoimetric equivalents of compounds **42** and **43** with palladium tetra(triphenylphosphine), sodium carbonate, glyme (DME)

followed by exposure to trifluoroacetic acid (TFA) per the methods described by Miller, J. Org. Chem., 50, 2121-2123 (1985). Subsequently, dimer **44** is formed by contacting at least 2 equivalents of compound **3a** with dibromo linker in the presence of an inert solvent such as dimethylformamide (DMF) and a base to scavenge the acid generated.

Scheme 2 found in FIG. 15 illustrates synthesis of estrogen receptor ligand **48** by conventional procedures described, for example, by Jones, et al., J. Med. Chem., 27(8):1057-1066 (1984). Specifically, methyl 4-hydroxybenzoate **45** is converted to compound **46** by first reacting an ω-chloroalkanol (where n is an integer of from 2 to 12) in an inert diluent such as tetrahydrofuran (THF) in the presence of triphenylphosphine (Ph_3P) and diethyl azodicarboxylate (DEAD) to provide for the ω-chloroalkoxy intermediate (not shown). Ester conversion to the free acid of the ω-chloroalkoxy intermediate occurs by contacting this compound with lithium hydroxide, in water and tetrahydrofuran (THF). Subsequently, the free acid is converted to the carboxylic acid chloride by reaction with $POCl_3$ in an inert diluent such as dichloromethane to provide for compound **46** where *n* is an integer of from 2 to 12.

At least stoichiometric amounts of compounds **46** and **47** are combined in a suitable inert solvent such as 1,2-dichloroethane comprising n-propanethiol and aluminum trichloride at approximately 0°C to provide for estrogen receptor ligand **48** having a reactive chloro group on the ω-chloroalkoxy substituent.

As also shown in FIG. 15, compound or ligand **48** can be used to prepare dimeric multibinding compunds **49** and **50** using conventional conditions described above.

Scheme 3 of FIG. 16 illustrates preparation of dimeric multibinding compounds **53** and **57** which, in turn, are prepared from estrogen receptor ligands **52** and **55** which are known in the art and described, for example, by

Lednicer, et al., J. Med. Chem., 12:881 (1969) and Tatee, et al., J. Med. Chem., 22:1509-1517 (1979). Specifically, the phenolic group of compound **51** is alkylated by reaction with at least a stoichiometric amount of 1,2-dibromoethane in an inert diluent such as acetone in the presence of potassium carbonate. The reaction is typically conducted at about 50°C for about 15 hours. Subsequently, demethylation of the resulting intermediate is achieved by conventional conditions including reaction with boron tribromide in an inert diluent such as methylene chloride to provide for compound **52**. Compound or ligand **52** can be used to prepare dimeric multibinding compound **53** using conventional conditions described above.

The reactions described above can be similarly conducted on compound **54** to provide compound **55** which can be used to prepare dimeric multibinding compound **56** using conventional conditions described above.

Further illustrations of the reactions set forth in FIG. 16 are found in FIG. 17 which illustrates synthesis of estrogen receptor ligand **58** which is disclosed, for example, by Crenshaw, et al., J. Med. Chem., 14:1185-1190 (1971). In turn, ligand **58** can be used to prepare dimeric multibinding compounds **59** and **61** by methods described above.

FIG. 18 illustrates reaction schemes to prepare dimeric multibinding compounds **62** and **63** using procedures described above.

Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which possess multibinding properties against estrogen receptor.

Specifically, factors such as the proper juxtaposition of the individual estrogen receptor ligands of a multibinding compound with respect to the

relevant array of binding sites on the estrogen receptor (target) is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for the estrogen receptor. These parameters include: (1) the identity of estrogen receptor ligand(s), (2) the orientation of ligands, (3) the valency of the construct or linker, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties against estrogen receptors (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

<u>Selection of ligand(s)</u>

A single estrogen receptor ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against estrogen receptors. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more

favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each estrogen receptor ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a crystal structure of a ligand bound to estrogen receptor allows one to identify one or more sites where linker attachment will not preclude the ligand:target interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely

selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in momomeric form.

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets.

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically inocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets and soluble receptor targets. In other instances where high-resolution structural information is not available, one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations

where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding sites reside on separate target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

<u>Linker geometry and rigidity.</u> The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

<u>Linker physical properties.</u> The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of

the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

Having chosen a set of *n* ligands (*n* being determined by the sum of the number of different attachment points for each ligand chosen) and *m* linkers by the process outlined above, a library of $(n!)m$ candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalies on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or

linker is attached to a solid support. Alternatively and preferably, the combinatorial libary is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties against estrogen receptor. Physical constants such as solubility under various solvent conditions and logD/clogD values can be determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data can be determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

In vitro efficacy, such as for receptor agonists and antagonists, can also be determined. Examples of such *in vitro* efficacy include assays for breast cancer using receptor binding in MCF-7 breast cell lysates, growth inhibition of MCR-7, growth inhibition of MCF-7 tamoxifen-resistant variants, and growth inhibition of human breast cancer cells in Courtenay-Mills clonogenic assay. *In vitro* assays for selective estrogen receptor modulator (SERM) activity include osteoclast-mediated bone resorption assays, estradiol-stimulated proliferation of primary cultures of rat long bone-derived osteoblasts, bone nodule formation

including deposition of collagen fibers and matrix mineralization, and alkaline phosphatase activity in Ishikawa human endometrial cancer cells.

In vivo assays can also be used including, by way of example, growth inhibition of MCF-7 in athymic mice, growth inhibition of MCF-7 in tamoxifen-resistant variants in athymic mice, and growth inhibition of MNU- and DMBA-induced mammary tumors in rats. *In vivo* assays for selective estrogen receptor modulator (SERM) activity include assays in ovariectomized rats to determine bone mineral density (single photonabsorptiometry), bone histomorphometry, serum osteocalcin levels, serum lipid levels and uterine weight as well as EnCa 101 human endometrial carcinoma in athymic mice.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libaries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the

structure and relative binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

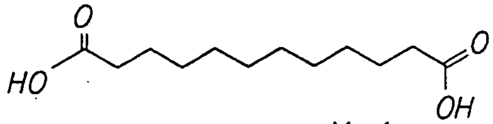
To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates,diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

COMPLEMENTARY BINDING CHEMISTRIES

First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-hydroxyamino
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_3$	amine
ketone	amine/$NaCNBH_3$	amine
amine	isocyanate	urea

Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below:

Diacids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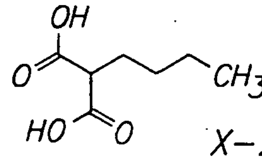
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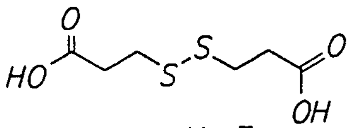
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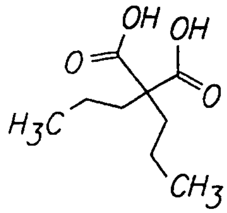
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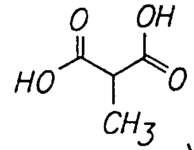
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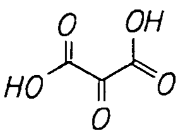
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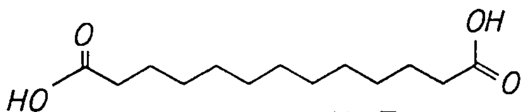
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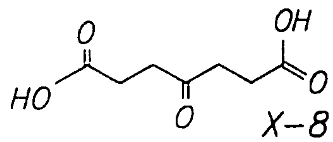
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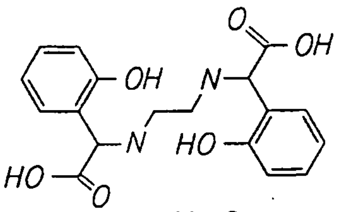
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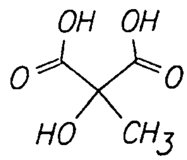
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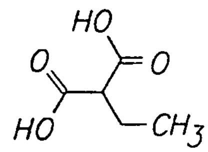
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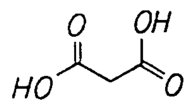
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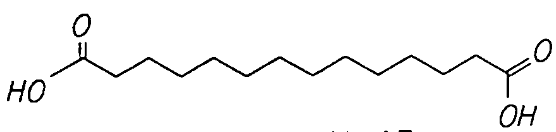
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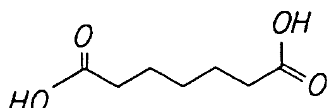
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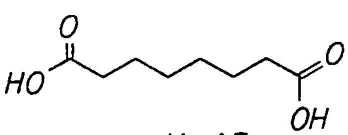
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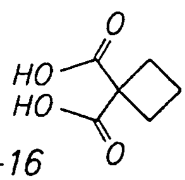
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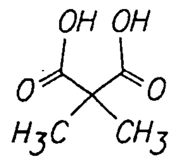
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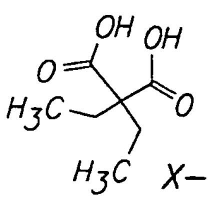
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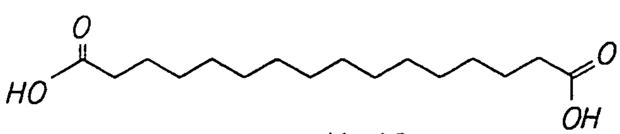
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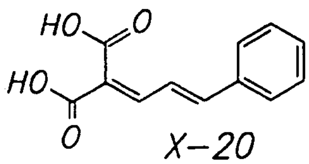

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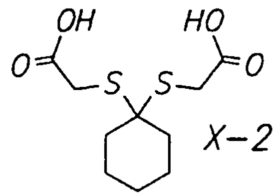

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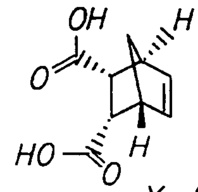

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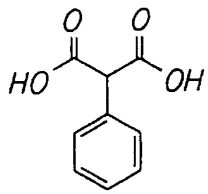

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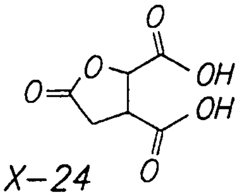

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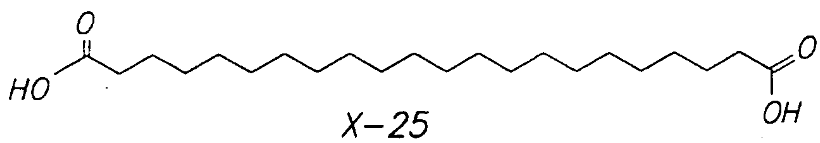

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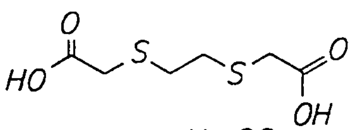

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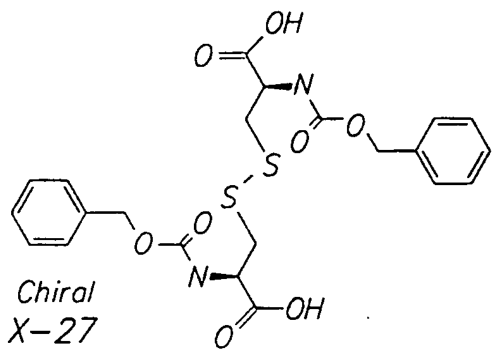

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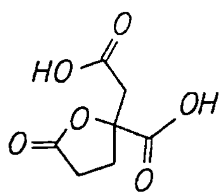

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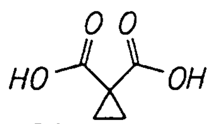

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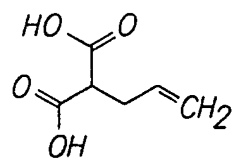

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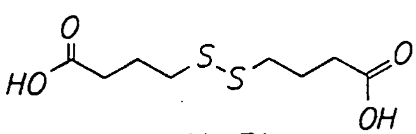

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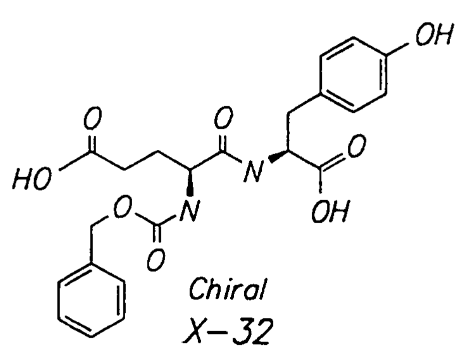

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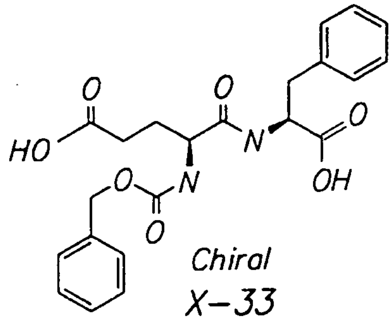

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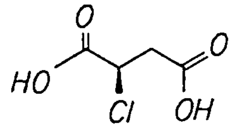

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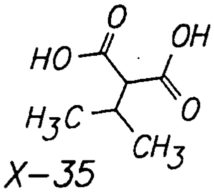

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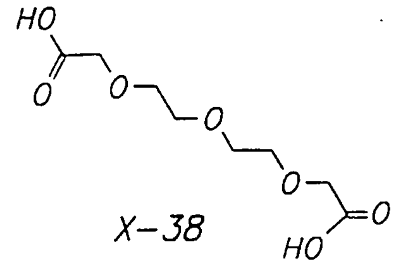

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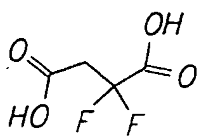

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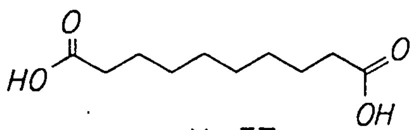

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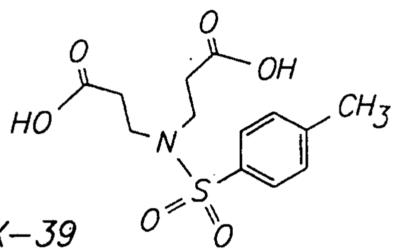

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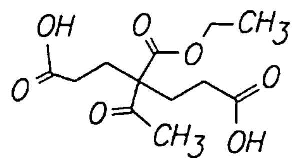

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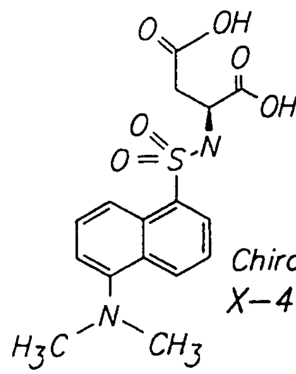

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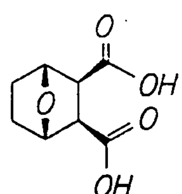

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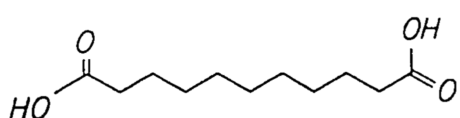

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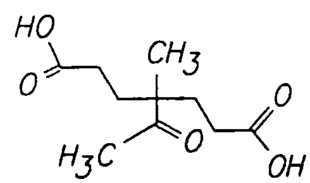

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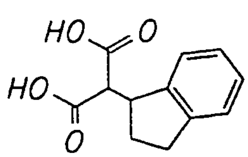

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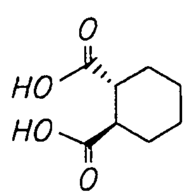

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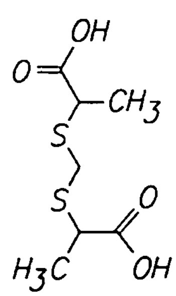

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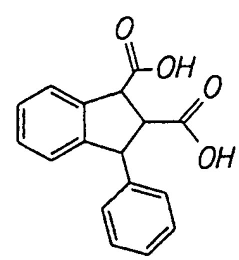

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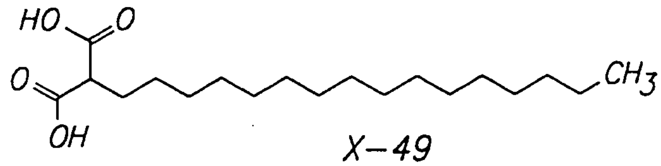

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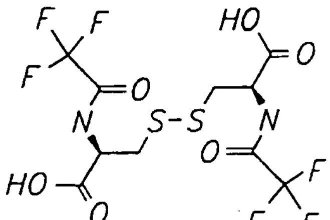

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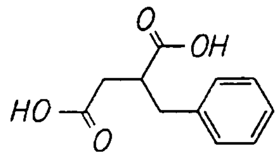

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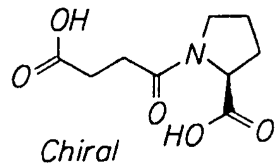

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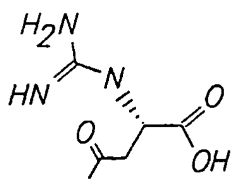

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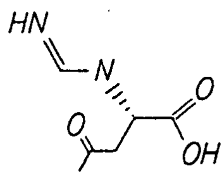

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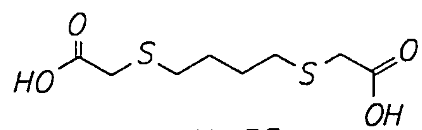

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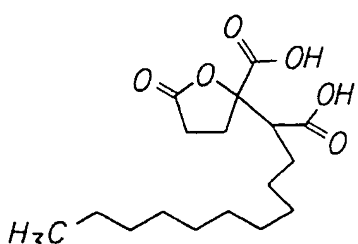

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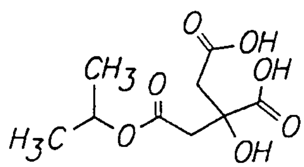

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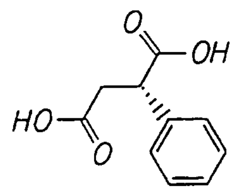

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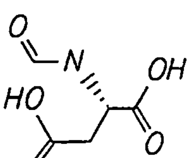

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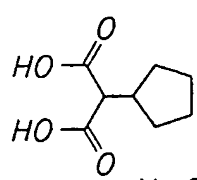

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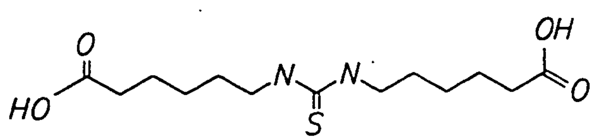

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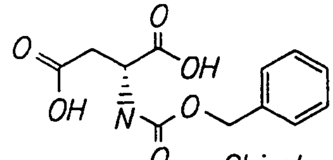

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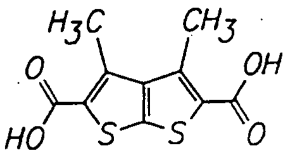
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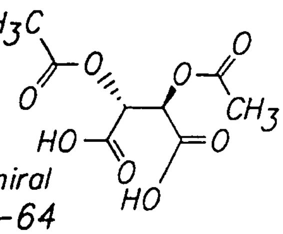
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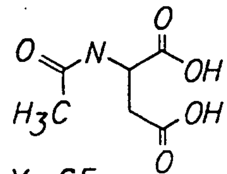
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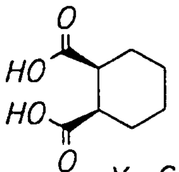
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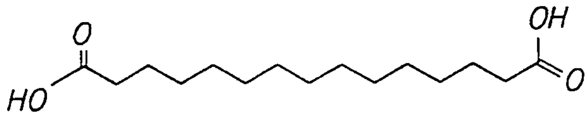
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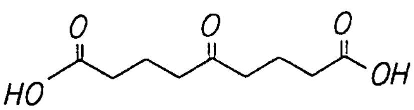
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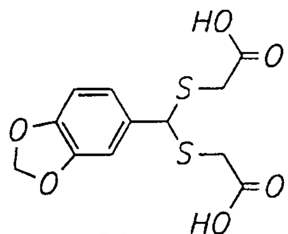
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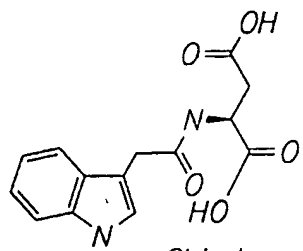
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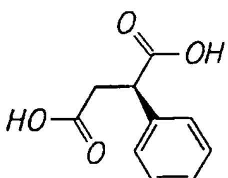
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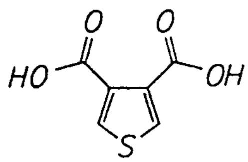
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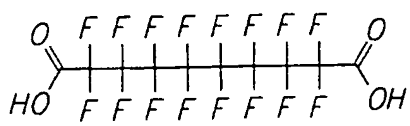
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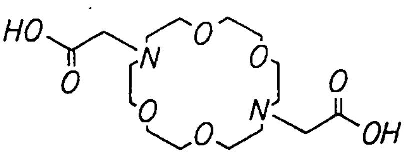
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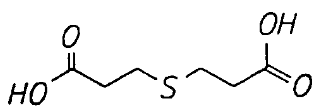
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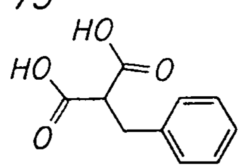
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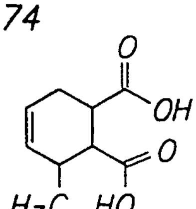
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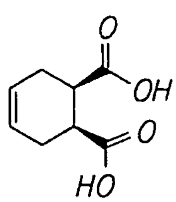
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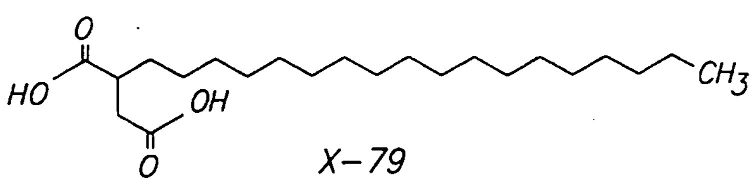
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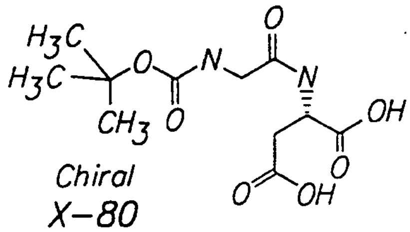

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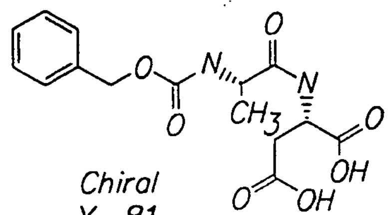

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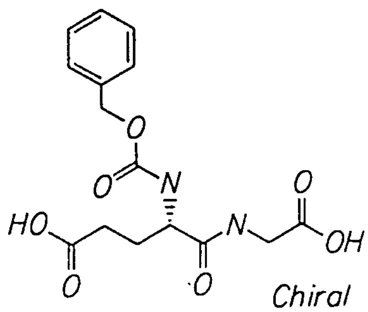

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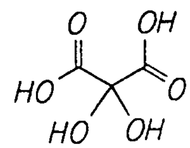

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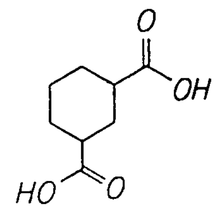

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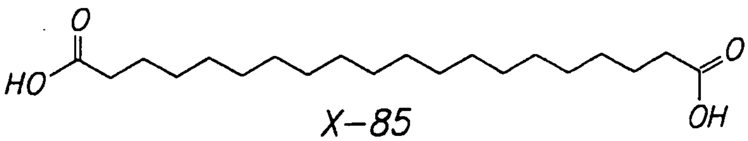

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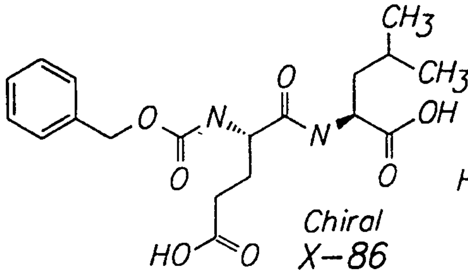

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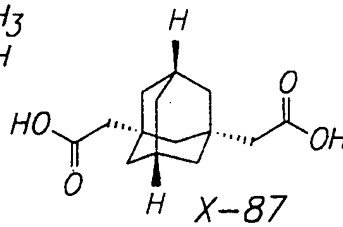

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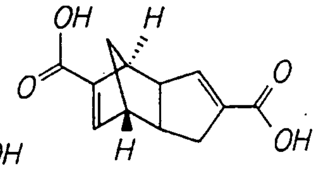

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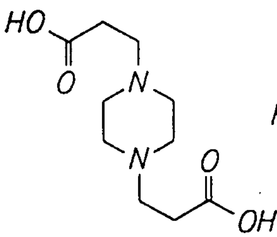

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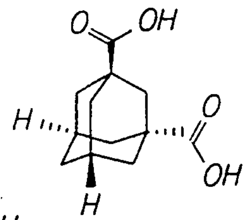

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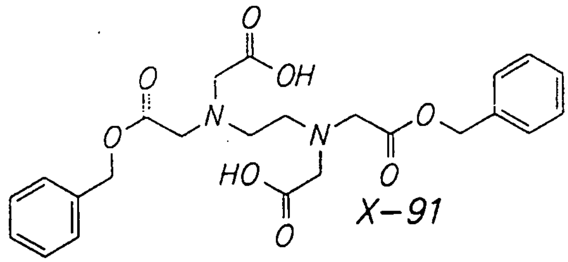

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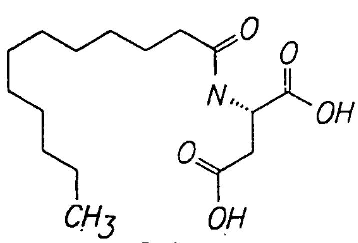

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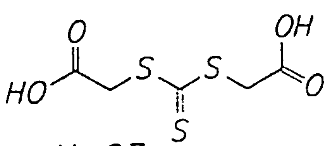

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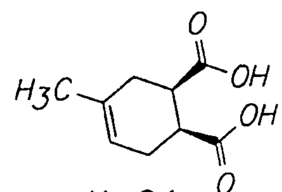

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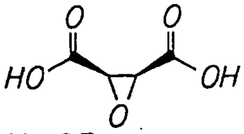

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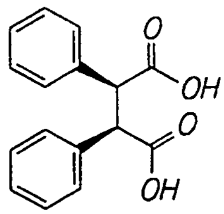

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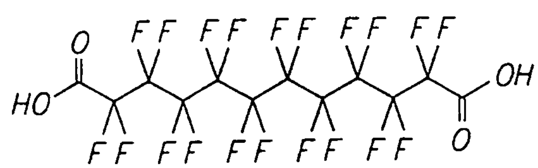

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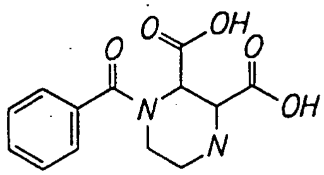

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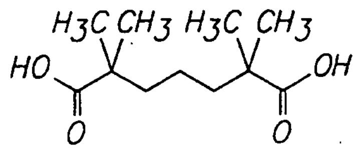

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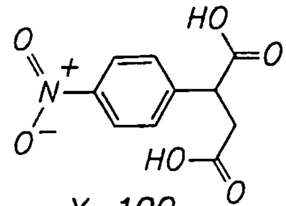

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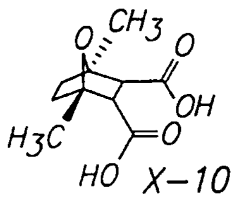

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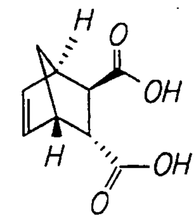

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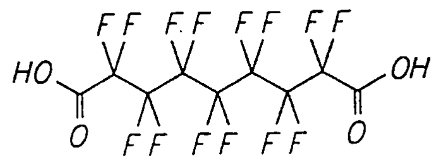

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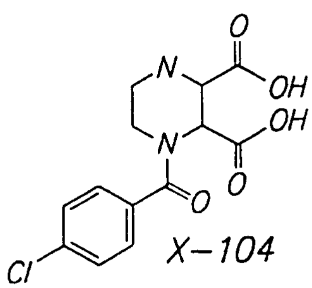

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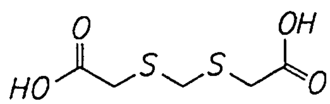

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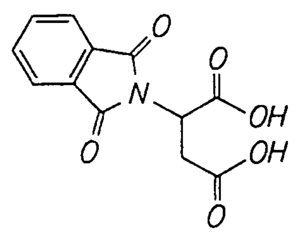

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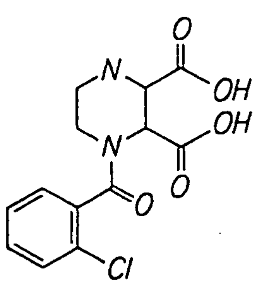

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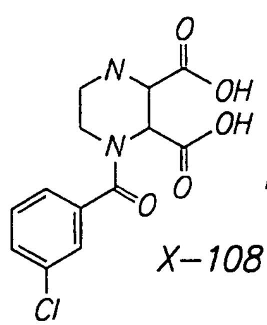

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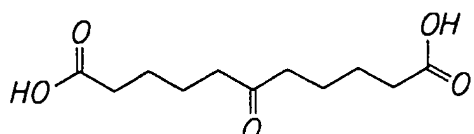

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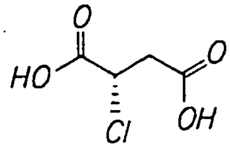
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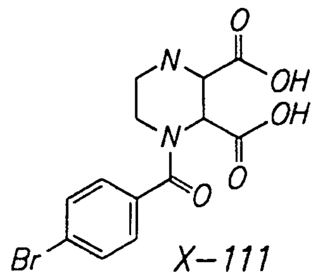
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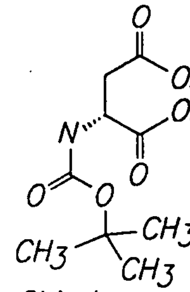
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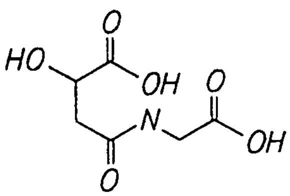
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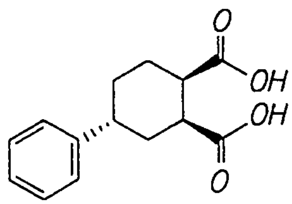
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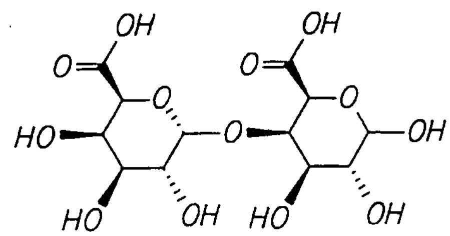
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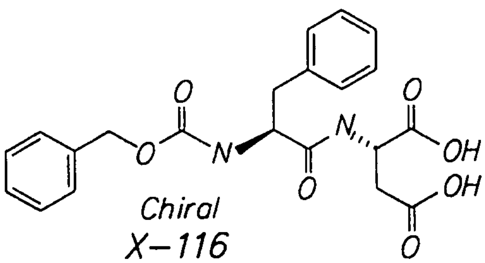
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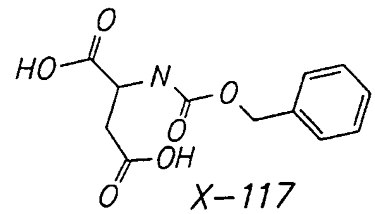
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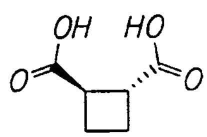
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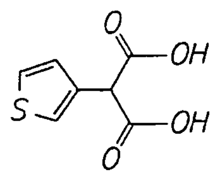
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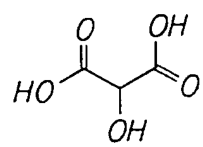
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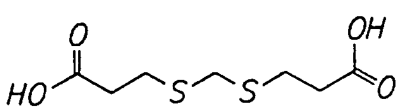
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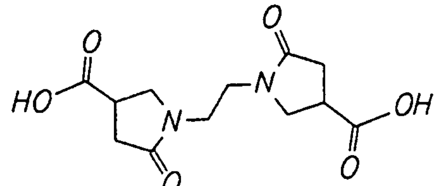
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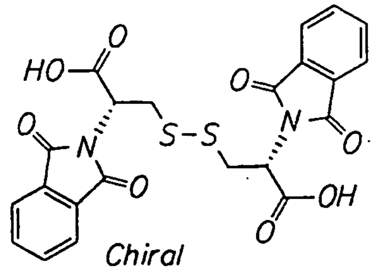
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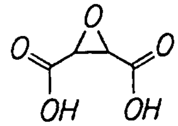
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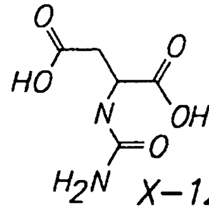
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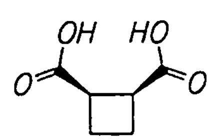
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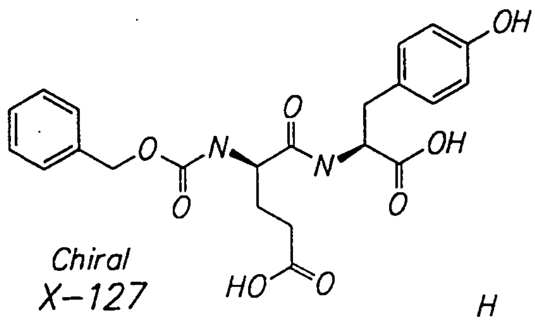

Chiral
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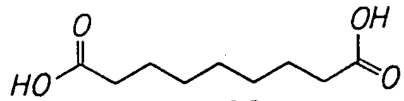

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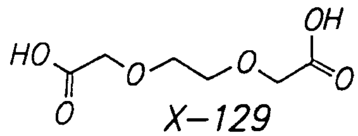

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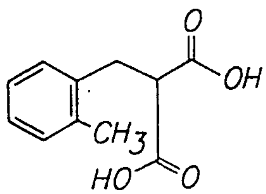

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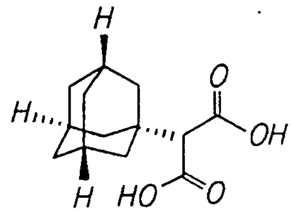

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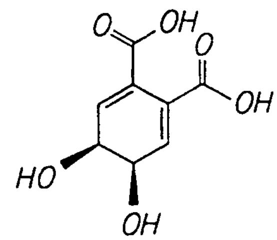

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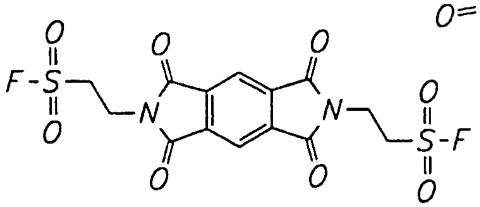
Disulfonyl Halides



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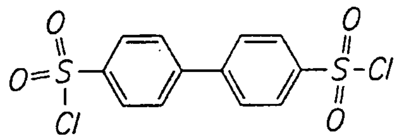

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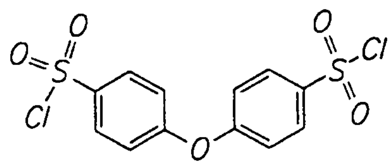

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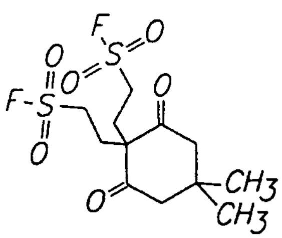

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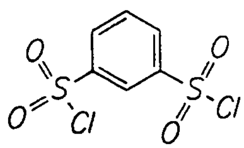

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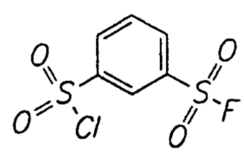

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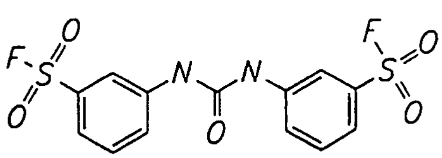

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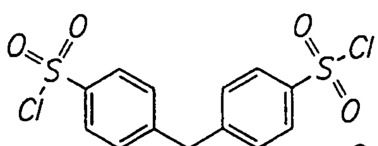

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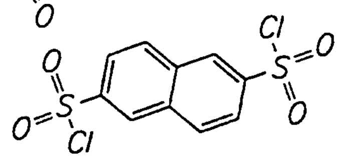

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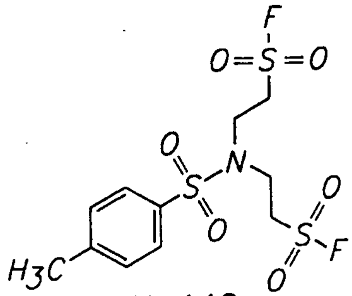

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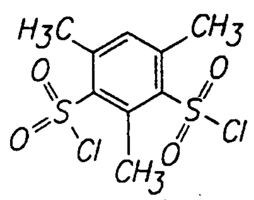

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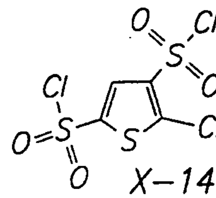

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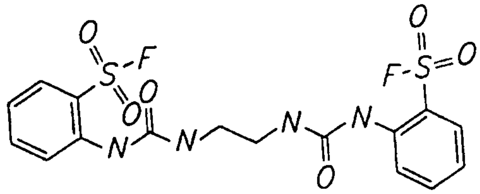

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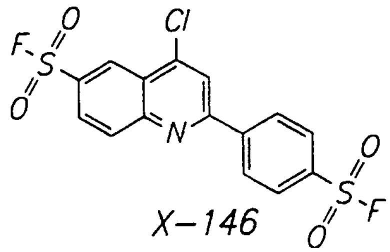

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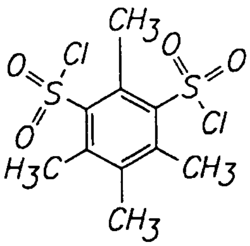

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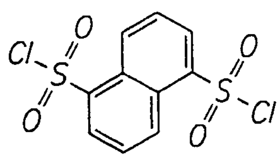

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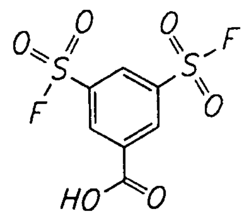

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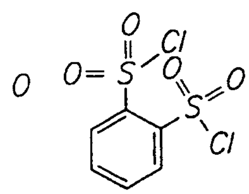

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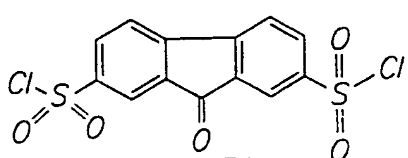

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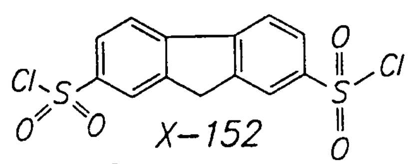

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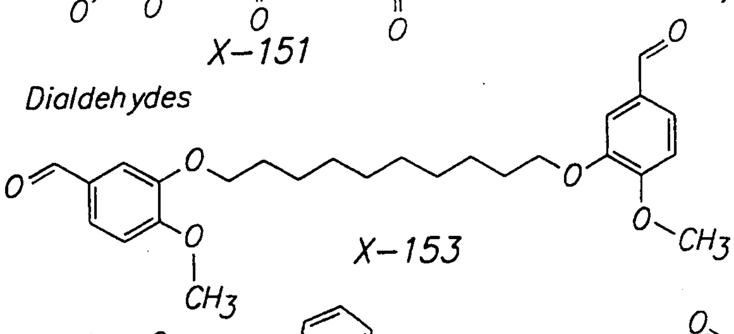
Dialdehydes



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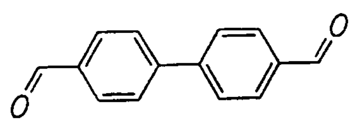

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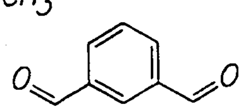

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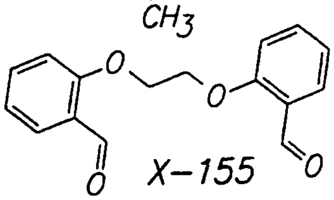

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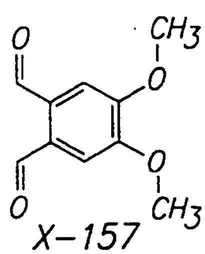

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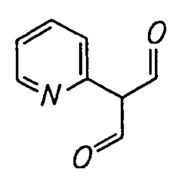

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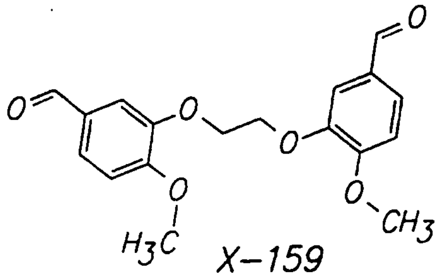
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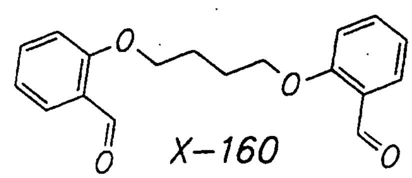
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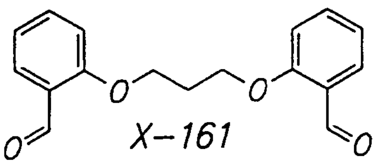
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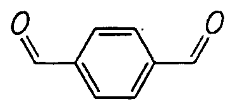
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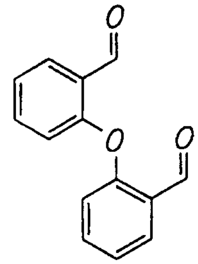
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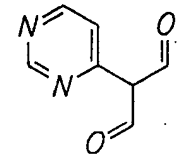
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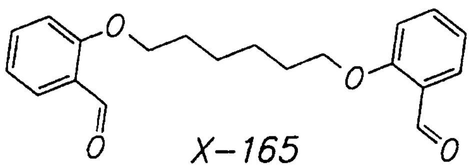
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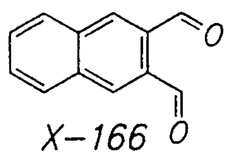
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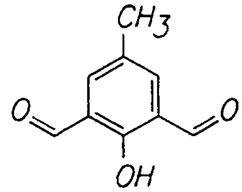
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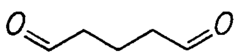
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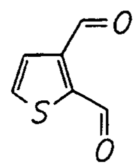
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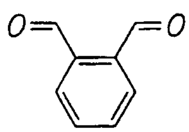
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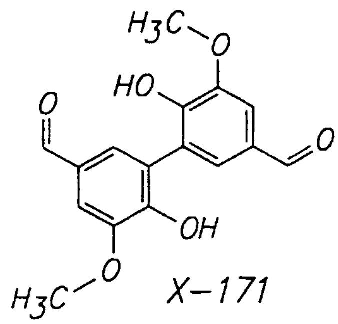
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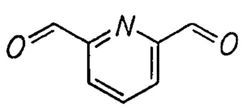
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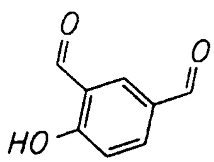
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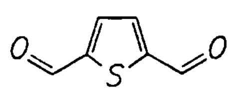
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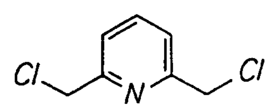
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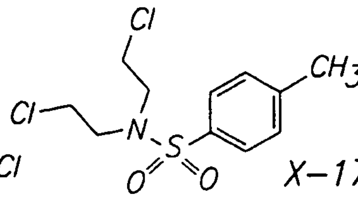
Dihalides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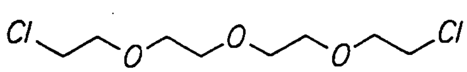
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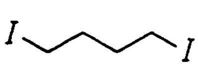
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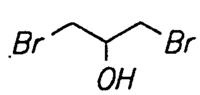
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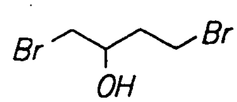
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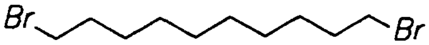
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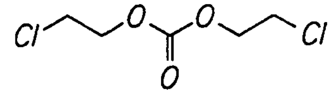
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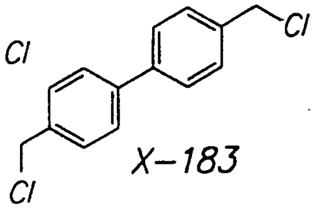
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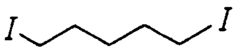
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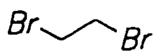
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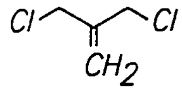
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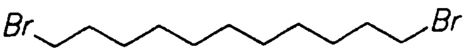
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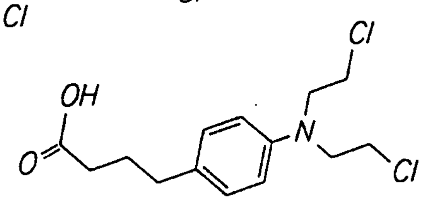
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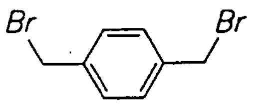
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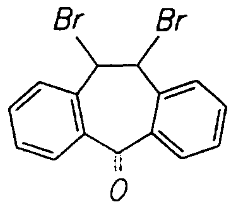
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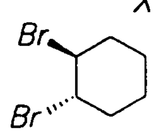
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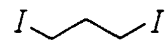
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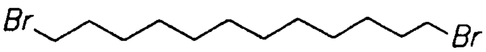
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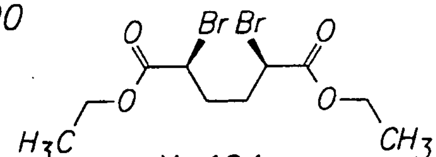
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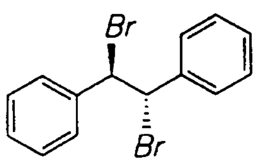
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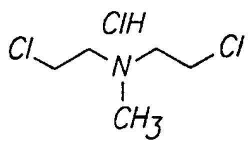
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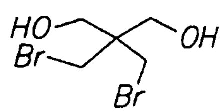
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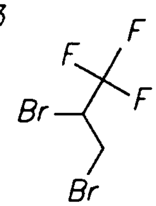
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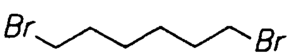
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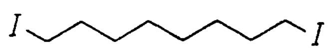
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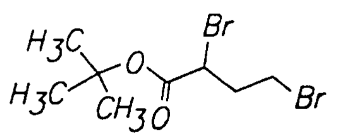
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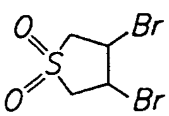
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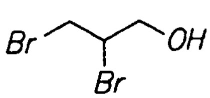
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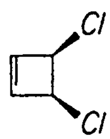
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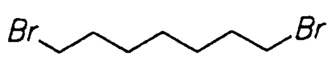
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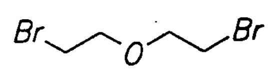
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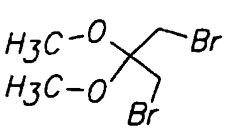
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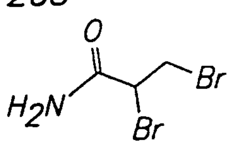
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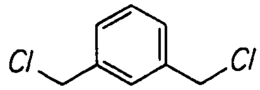
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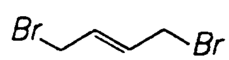
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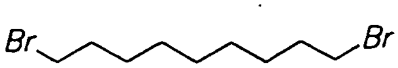
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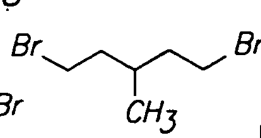
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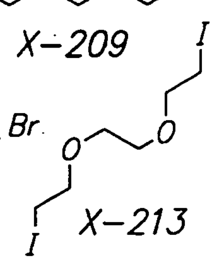
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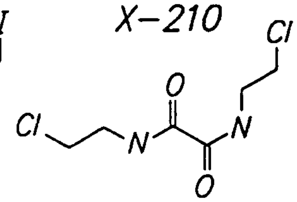
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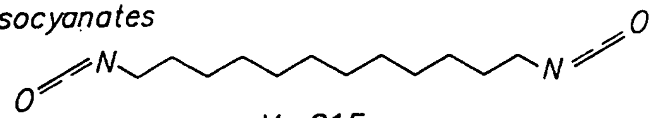

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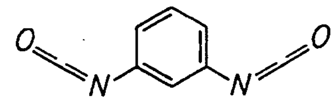

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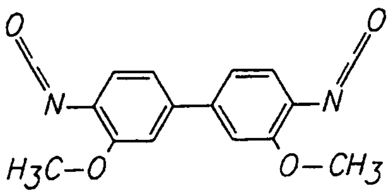

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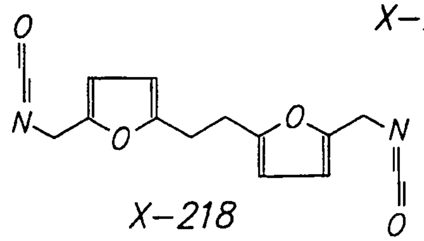

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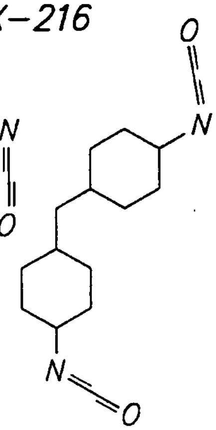

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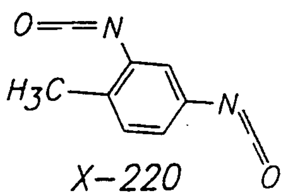

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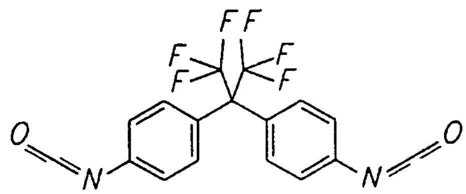

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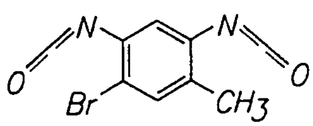

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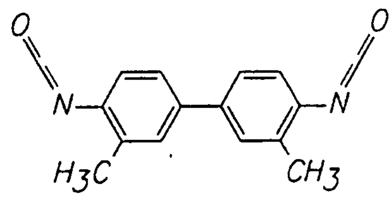

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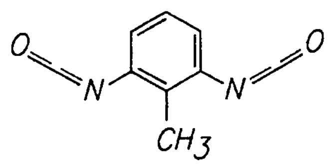

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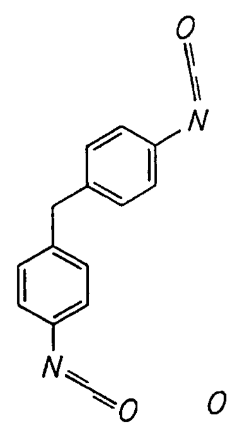

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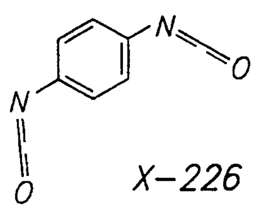

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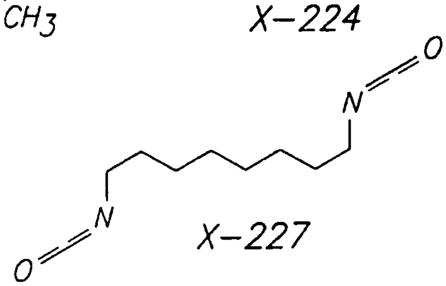

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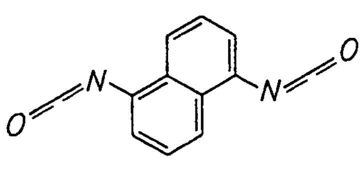

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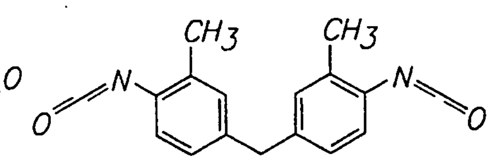

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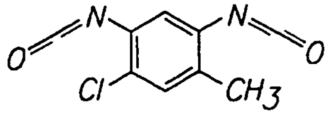

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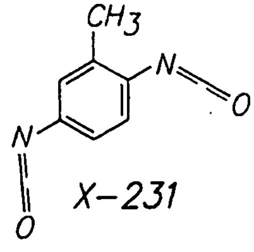

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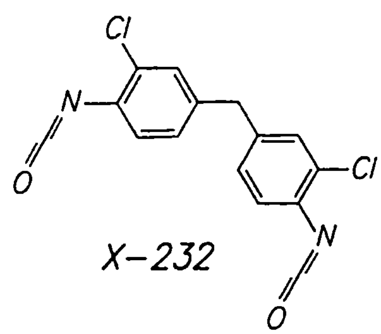

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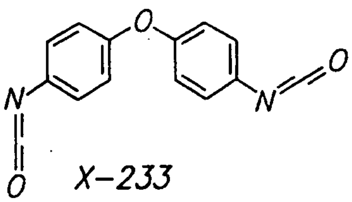

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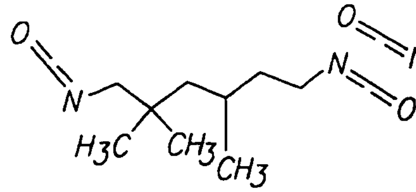

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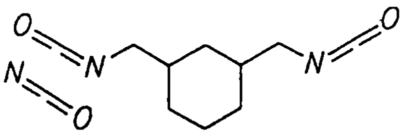

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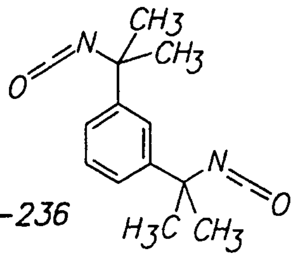

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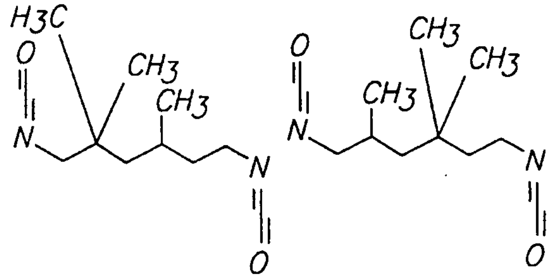

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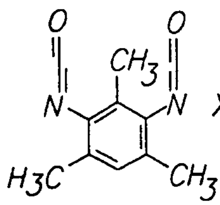

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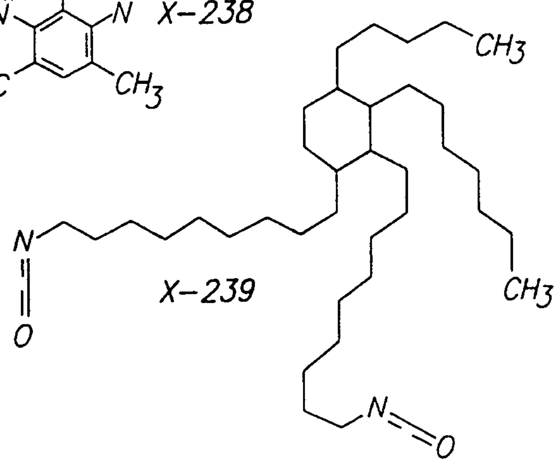

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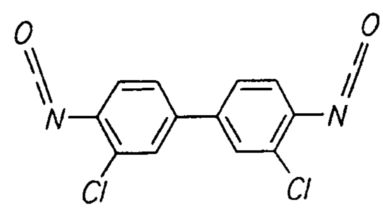

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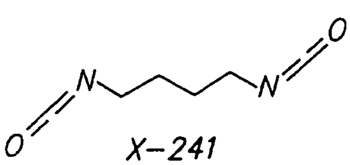

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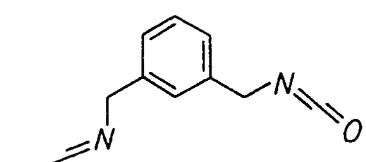

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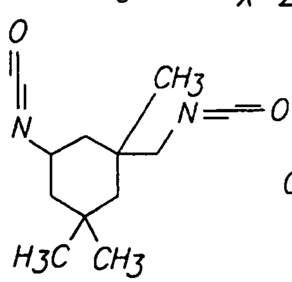

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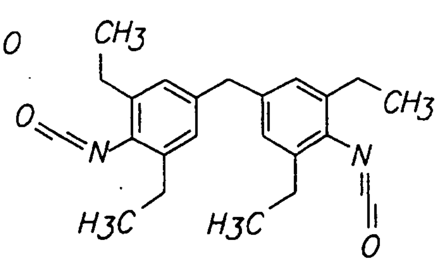

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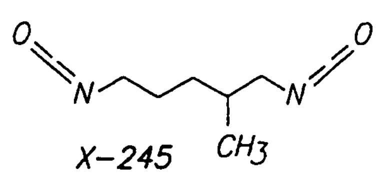

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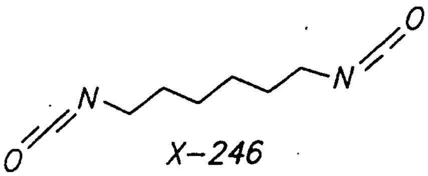

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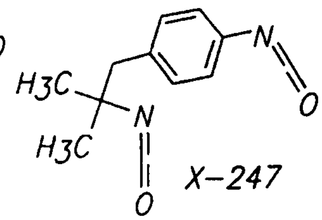

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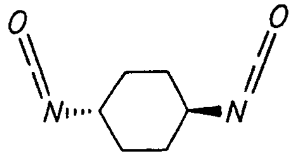

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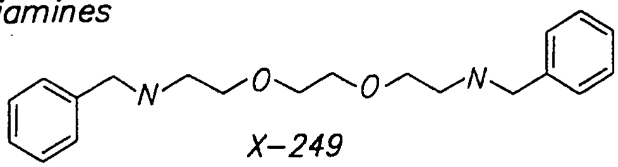

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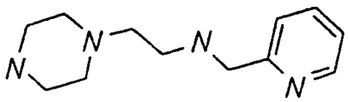

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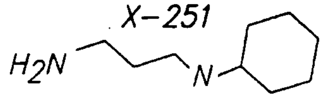

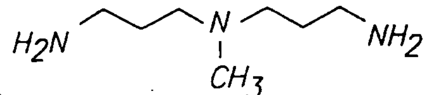

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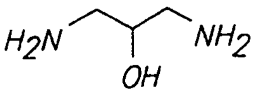

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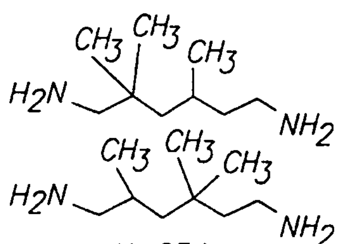

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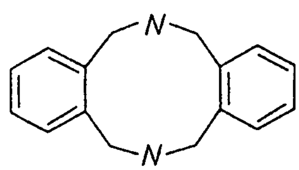

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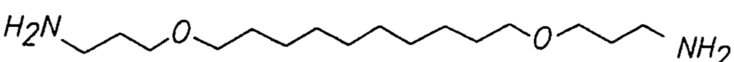

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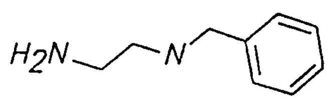

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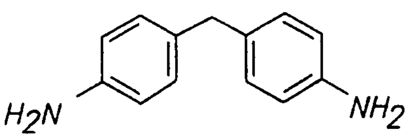

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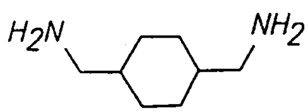

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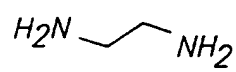

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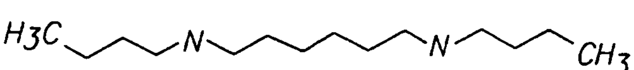

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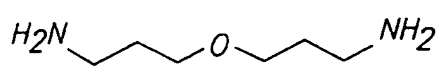

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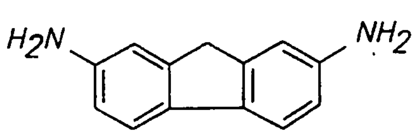

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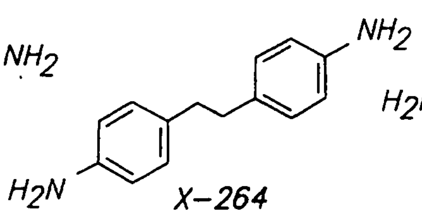

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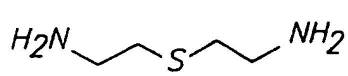

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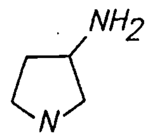

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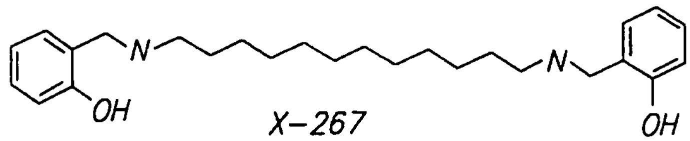

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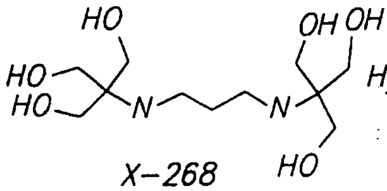

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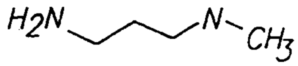

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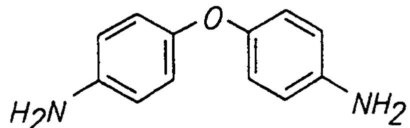

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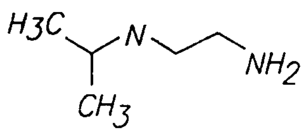

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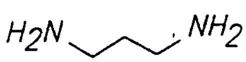

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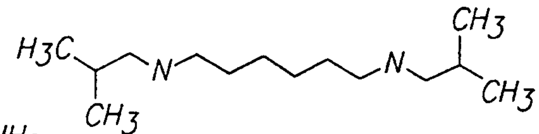

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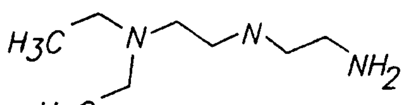

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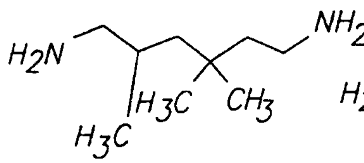

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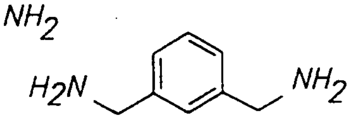

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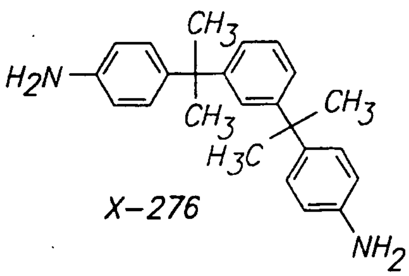

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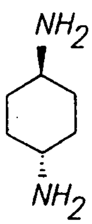

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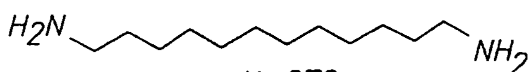

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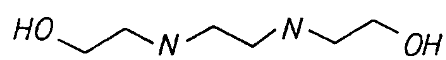

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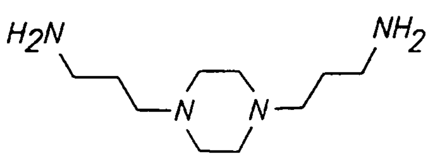

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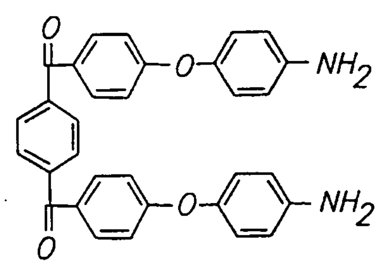

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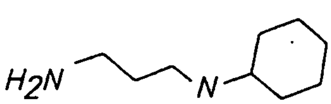

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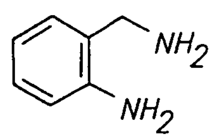

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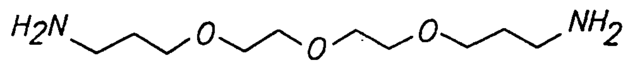

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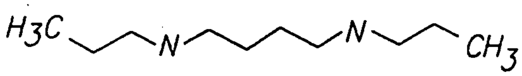

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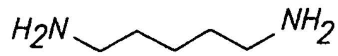

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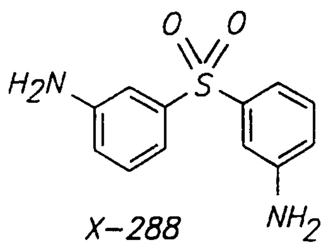

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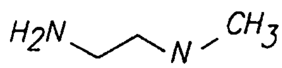

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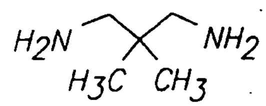

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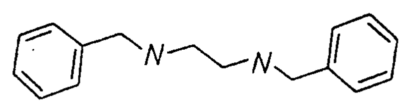

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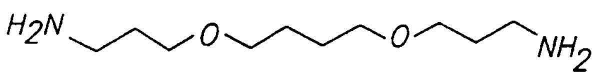

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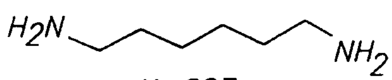

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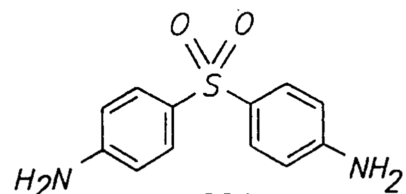

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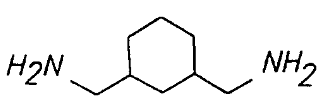

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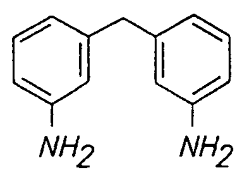

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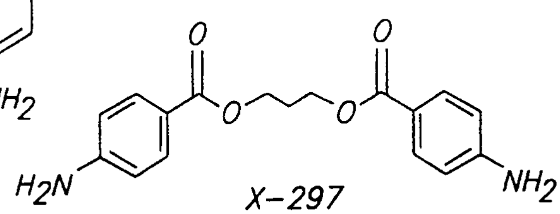

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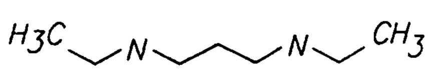

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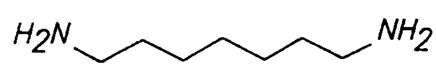

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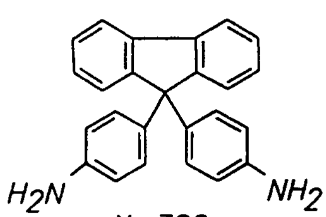

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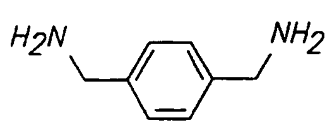

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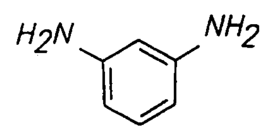

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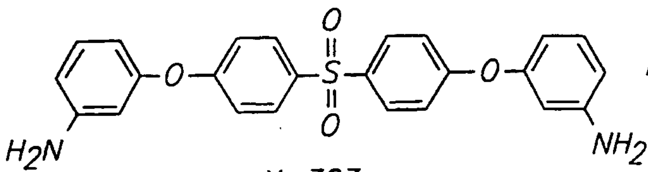

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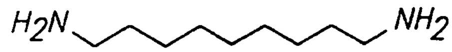

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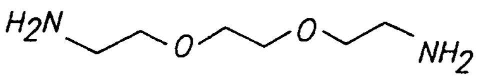

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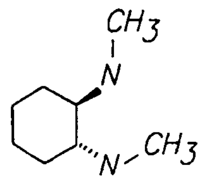

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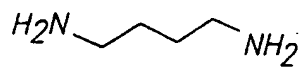

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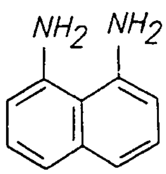

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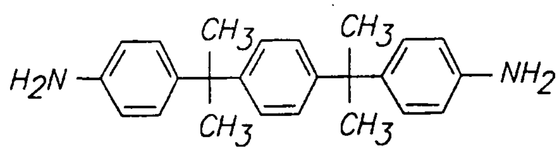

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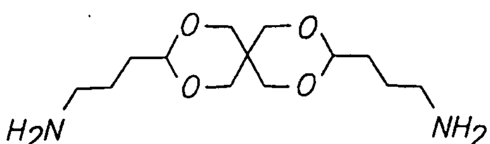

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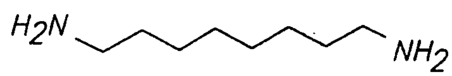

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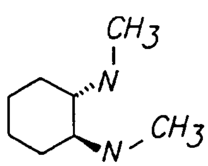

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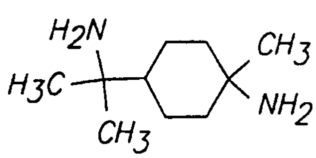

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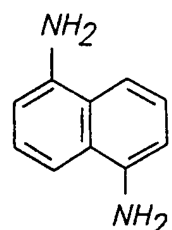

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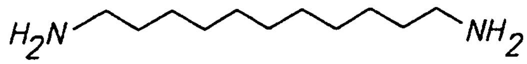

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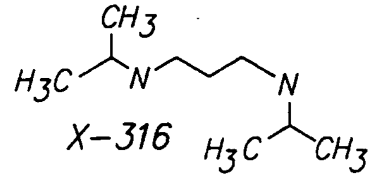

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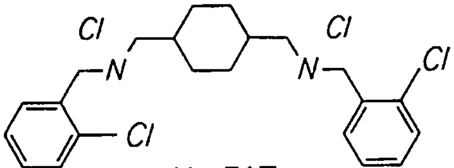

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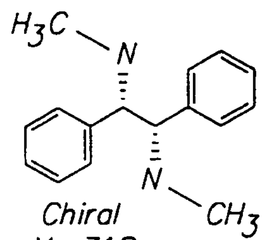

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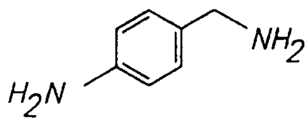

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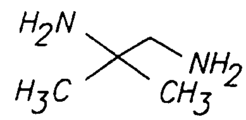

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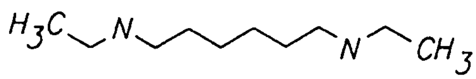

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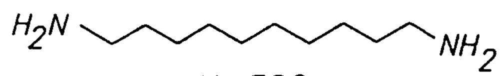

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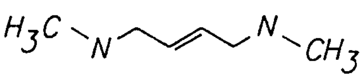

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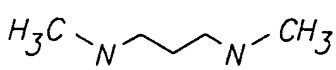

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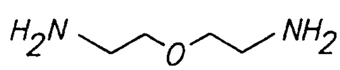

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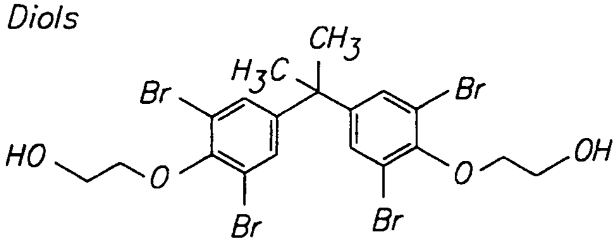

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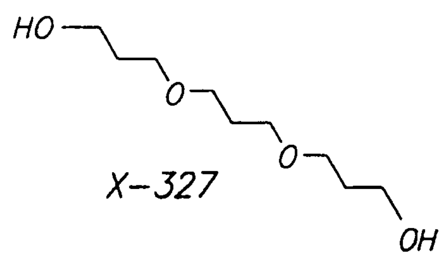

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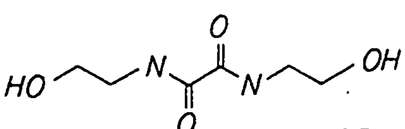

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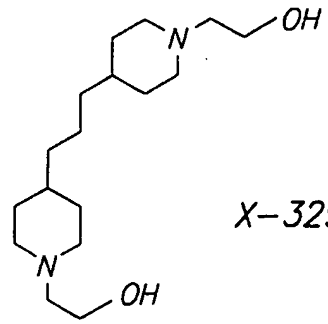

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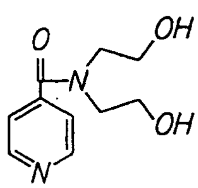

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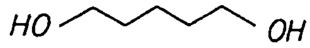

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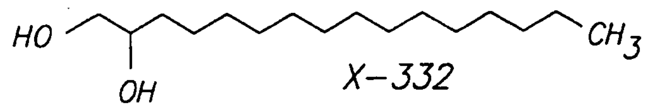

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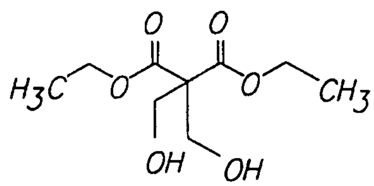

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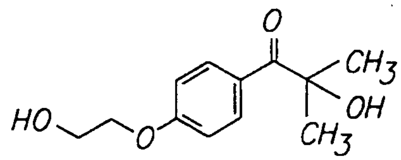

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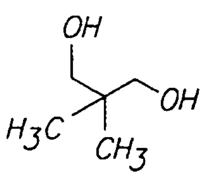

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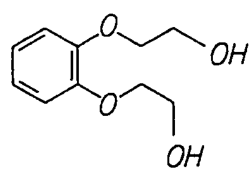

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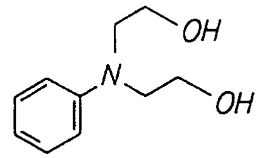

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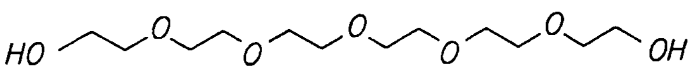

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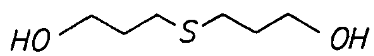

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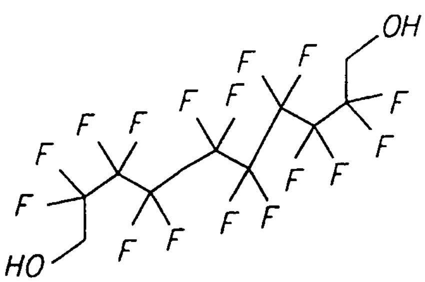

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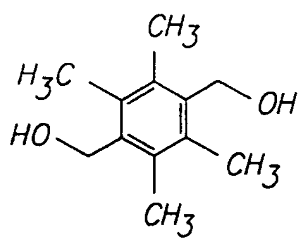

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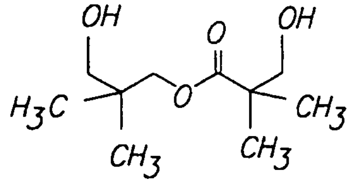

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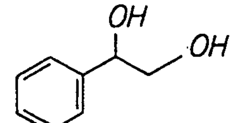

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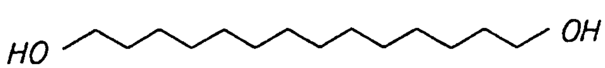

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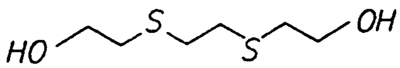

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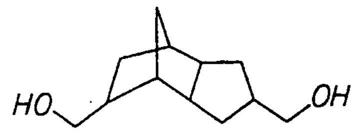

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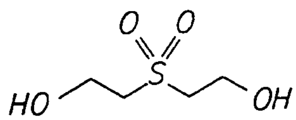

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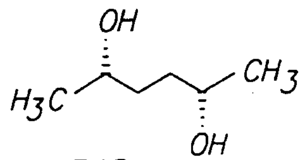

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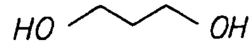

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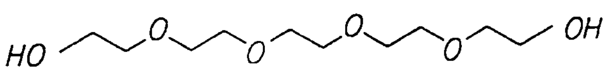

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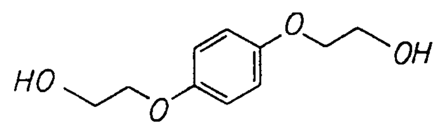

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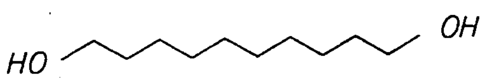

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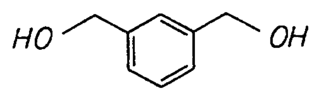

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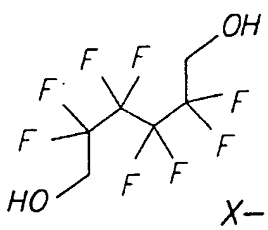

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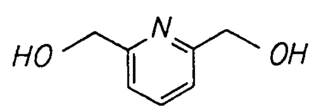

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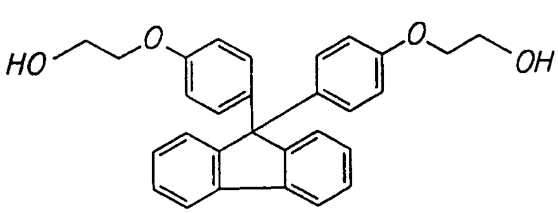

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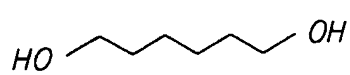

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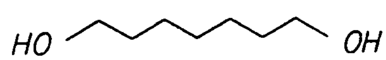

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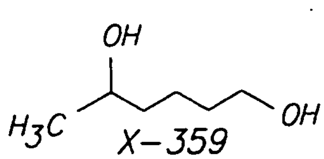

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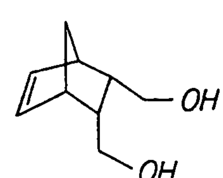

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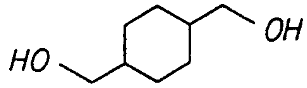

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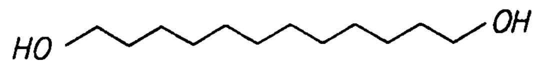

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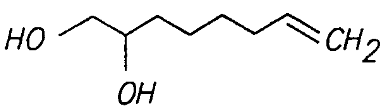

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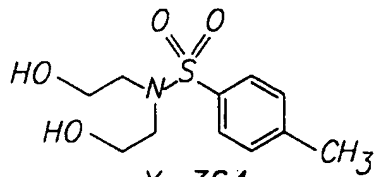

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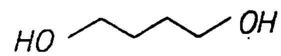

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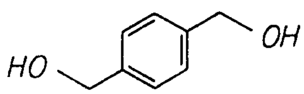

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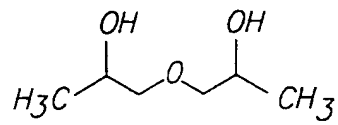

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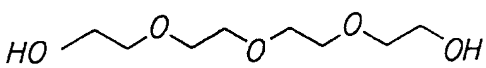

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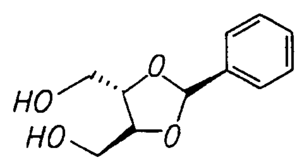

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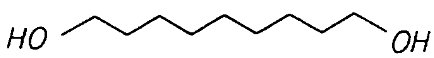

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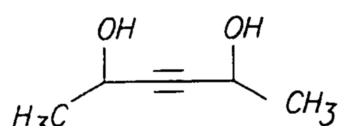

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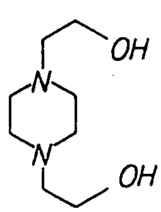

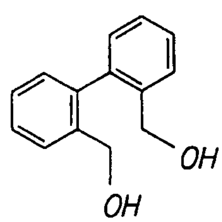

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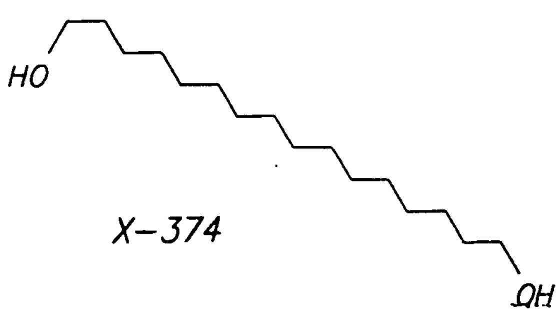

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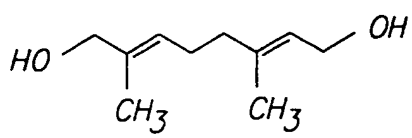

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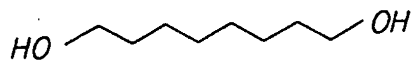

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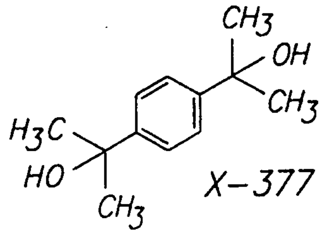

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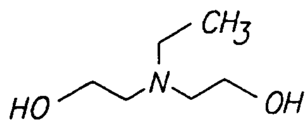

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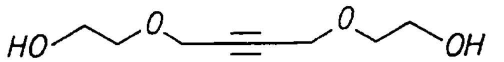

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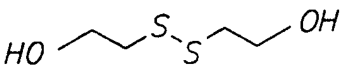

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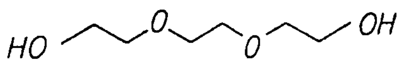

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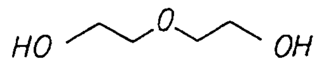

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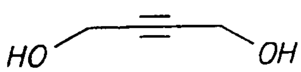

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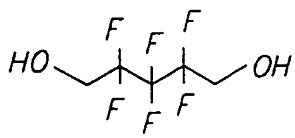

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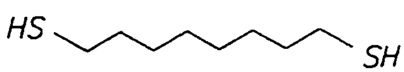

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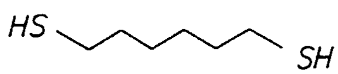

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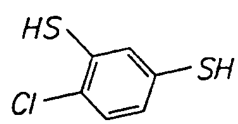

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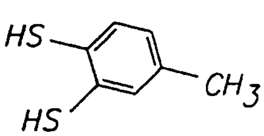

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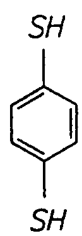

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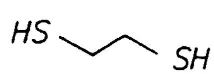

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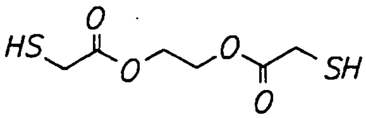
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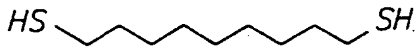
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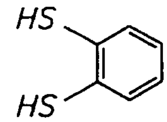
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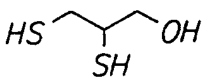
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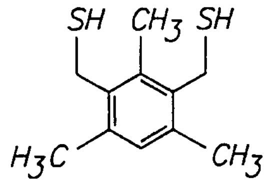
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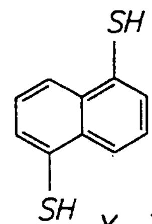
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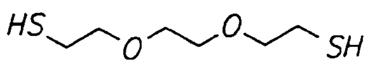
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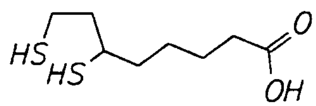
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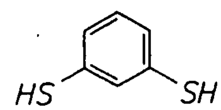
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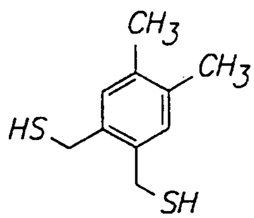
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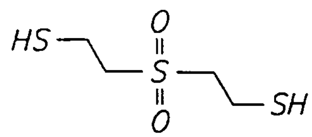
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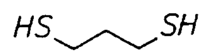
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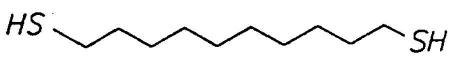
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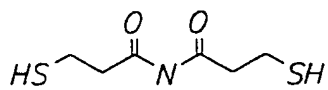
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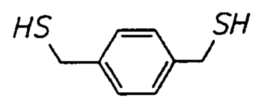
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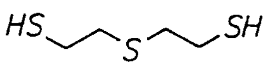
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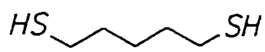
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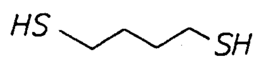
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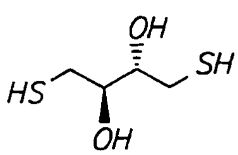
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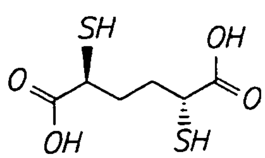
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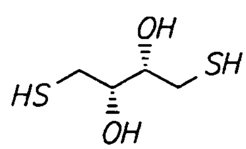
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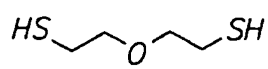
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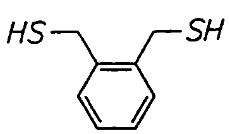
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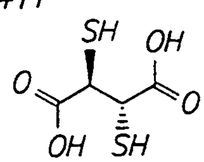
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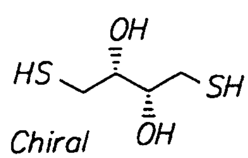
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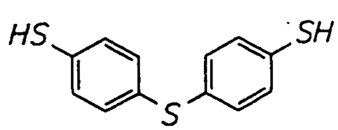
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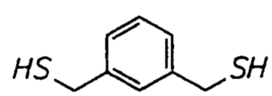
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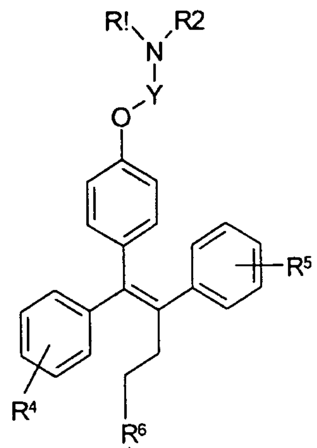
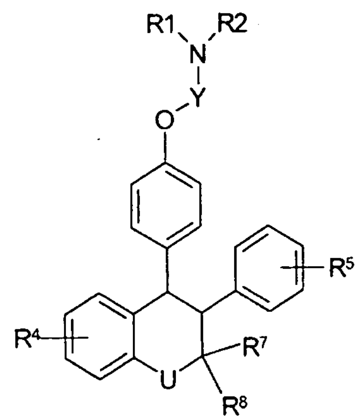
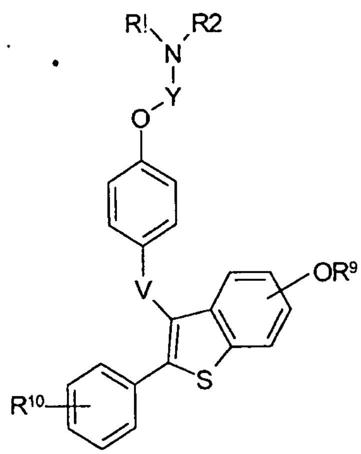
Representative ligands for use in this invention include, by way of example, L-1 through L-3 as follows:



L-1



L-2



L-3

where R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9, R^{10} U, V and Y are as defined above.

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-3 above and the second ligand and linker is selected from the following:

L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-
L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-
L-1/X-109-	L-1/X-110-	L-1/X-111-	L-1/X-112-	L-1/X-113-	L-1/X-114-
L-1/X-115-	L-1/X-116-	L-1/X-117-	L-1/X-118-	L-1/X-119-	L-1/X-120-
L-1/X-121-	L-1/X-122-	L-1/X-123-	L-1/X-124-	L-1/X-125-	L-1/X-126-
L-1/X-127-	L-1/X-128-	L-1/X-129-	L-1/X-130-	L-1/X-131-	L-1/X-132-
L-1/X-133-	L-1/X-134-	L-1/X-135-	L-1/X-136-	L-1/X-137-	L-1/X-138-
L-1/X-139-	L-1/X-140-	L-1/X-141-	L-1/X-142-	L-1/X-143-	L-1/X-144-
L-1/X-145-	L-1/X-146-	L-1/X-147-	L-1/X-148-	L-1/X-149-	L-1/X-150-
L-1/X-151-	L-1/X-152-	L-1/X-153-	L-1/X-154-	L-1/X-155-	L-1/X-156-
L-1/X-157-	L-1/X-158-	L-1/X-159-	L-1/X-160-	L-1/X-161-	L-1/X-162-
L-1/X-163-	L-1/X-164-	L-1/X-165-	L-1/X-166-	L-1/X-167-	L-1/X-168-
L-1/X-169-	L-1/X-170-	L-1/X-171-	L-1/X-172-		
L-1/X-173-	L-1/X-174-	L-1/X-175-	L-1/X-176-	L-1/X-177-	L-1/X-178-
L-1/X-179-	L-1/X-180-	L-1/X-181-	L-1/X-182-	L-1/X-183-	L-1/X-184-
L-1/X-185-	L-1/X-186-	L-1/X-187-	L-1/X-188-	L-1/X-189-	L-1/X-190-
L-1/X-191-	L-1/X-192-	L-1/X-193-	L-1/X-194-	L-1/X-195-	L-1/X-196-
L-1/X-197-	L-1/X-198-	L-1/X-199-	L-1/X-200-	L-1/X-201-	L-1/X-202-
L-1/X-203-	L-1/X-204-	L-1/X-205-	L-1/X-206-	L-1/X-207-	L-1/X-208-
L-1/X-209-	L-1/X-210-	L-1/X-211-	L-1/X-212-	L-1/X-213-	L-1/X-214-
L-1/X-215-	L-1/X-216-	L-1/X-217-	L-1/X-218-	L-1/X-219-	L-1/X-220-
L-1/X-221-	L-1/X-222-	L-1/X-223-	L-1/X-224-	L-1/X-225-	L-1/X-226-
L-1/X-227-	L-1/X-228-	L-1/X-229-	L-1/X-230-	L-1/X-231-	L-1/X-232-
L-1/X-233-	L-1/X-234-	L-1/X-235-	L-1/X-236-	L-1/X-237-	L-1/X-238-
L-1/X-239-	L-1/X-240-	L-1/X-241-	L-1/X-242-	L-1/X-243-	L-1/X-244-
L-1/X-245-	L-1/X-246-	L-1/X-247-	L-1/X-248-	L-1/X-249-	L-1/X-250-
L-1/X-251-	L-1/X-252-	L-1/X-253-	L-1/X-254-	L-1/X-255-	L-1/X-256-
L-1/X-257-	L-1/X-258-	L-1/X-259-	L-1/X-260-	L-1/X-261-	L-1/X-262-

L-1/X-263-	L-1/X-264-	L-1/X-265-	L-1/X-266-	L-1/X-267-	L-1/X-268-
L-1/X-269-	L-1/X-270-	L-1/X-271-	L-1/X-272-	L-1/X-273-	L-1/X-274-
L-1/X-275-	L-1/X-276-	L-1/X-277-	L-1/X-278-	L-1/X-279-	L-1/X-280-
L-1/X-281-	L-1/X-282-	L-1/X-283-	L-1/X-284-	L-1/X-285-	L-1/X-286-
L-1/X-287-	L-1/X-288-	L-1/X-289-	L-1/X-290-	L-1/X-291-	L-1/X-292-
L-1/X-293-	L-1/X-294-	L-1/X-295-	L-1/X-296-	L-1/X-297-	L-1/X-298-
L-1/X-299-	L-1/X-300-	L-1/X-301-	L-1/X-302-	L-1/X-303-	L-1/X-304-
L-1/X-305-	L-1/X-306-	L-1/X-307-	L-1/X-308-	L-1/X-309-	L-1/X-310-
L-1/X-311-	L-1/X-312-	L-1/X-313-	L-1/X-314-	L-1/X-315-	L-1/X-316-
L-1/X-317-	L-1/X-318-	L-1/X-319-	L-1/X-320-	L-1/X-321-	L-1/X-322-
L-1/X-323-	L-1/X-324-	L-1/X-325-	L-1/X-326-	L-1/X-327-	L-1/X-328-
L-1/X-329-	L-1/X-330-	L-1/X-331-	L-1/X-332-	L-1/X-333-	L-1/X-334-
L-1/X-335-	L-1/X-336-	L-1/X-337-	L-1/X-338-	L-1/X-339-	L-1/X-340-
L-1/X-341-	L-1/X-342-	L-1/X-343-	L-1/X-344-	L-1/X-345-	L-1/X-346-
L-1/X-347-	L-1/X-348-	L-1/X-349-	L-1/X-350-	L-1/X-351-	L-1/X-352-
L-1/X-353-	L-1/X-354-	L-1/X-355-	L-1/X-356-	L-1/X-357-	L-1/X-358-
L-1/X-359-	L-1/X-360-	L-1/X-361-	L-1/X-362-	L-1/X-363-	L-1/X-364-
L-1/X-365-	L-1/X-366-	L-1/X-367-	L-1/X-368-	L-1/X-369-	L-1/X-370-
L-1/X-371-	L-1/X-372-	L-1/X-373-	L-1/X-374-	L-1/X-375-	L-1/X-376-
L-1/X-377-	L-1/X-378-	L-1/X-379-	L-1/X-380-	L-1/X-381-	L-1/X-382-
L-1/X-383-	L-1/X-384-	L-1/X-385-	L-1/X-386-	L-1/X-387-	L-1/X-388-
L-1/X-389-	L-1/X-390-	L-1/X-391-	L-1/X-392-	L-1/X-393-	L-1/X-394-
L-1/X-395-	L-1/X-396-	L-1/X-397-	L-1/X-398-	L-1/X-399-	L-1/X-400-
L-1/X-401-	L-1/X-402-	L-1/X-403-	L-1/X-404-	L-1/X-405-	L-1/X-406-
L-1/X-407-	L-1/X-408-	L-1/X-409-	L-1/X-410-	L-1/X-411-	L-1/X-412-
L-1/X-413-	L-1/X-414-	L-1/X-415-	L-1/X-416-	L-1/X-417-	L-1/X-418-

L-2/X-1-	L-2/X-2-	L-2/X-3-	L-2/X-4-	L-2/X-5-	L-2/X-6-
L-2/X-7-	L-2/X-8-	L-2/X-9-	L-2/X-10-	L-2/X-11-	L-2/X-12-
L-2/X-13-	L-2/X-14-	L-2/X-15-	L-2/X-16-	L-2/X-17-	L-2/X-18-
L-2/X-19-	L-2/X-20-	L-2/X-21-	L-2/X-22-	L-2/X-23-	L-2/X-24-
L-2/X-25-	L-2/X-26-	L-2/X-27-	L-2/X-28-	L-2/X-29-	L-2/X-30-
L-2/X-31-	L-2/X-32-	L-2/X-33-	L-2/X-34-	L-2/X-35-	L-2/X-36-
L-2/X-37-	L-2/X-38-	L-2/X-39-	L-2/X-40-	L-2/X-41-	L-2/X-42-
L-2/X-43-	L-2/X-44-	L-2/X-45-	L-2/X-46-	L-2/X-47-	L-2/X-48-
L-2/X-49-	L-2/X-50-	L-2/X-51-	L-2/X-52-	L-2/X-53-	L-2/X-54-
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L-2/X-97-	L-2/X-98-	L-2/X-99-	L-2/X-100-	L-2/X-101-	L-2/X-102-
L-2/X-103-	L-2/X-104-	L-2/X-105-	L-2/X-106-	L-2/X-107-	L-2/X-108-
L-2/X-109-	L-2/X-110-	L-2/X-111-	L-2/X-112-	L-2/X-113-	L-2/X-114-

L-2/X-115-	L-2/X-116-	L-2/X-117-	L-2/X-118-	L-2/X-119-	L-2/X-120-
L-2/X-121-	L-2/X-122-	L-2/X-123-	L-2/X-124-	L-2/X-125-	L-2/X-126-
L-2/X-127-	L-2/X-128-	L-2/X-129-	L-2/X-130-	L-2/X-131-	L-2/X-132-
L-2/X-133-	L-2/X-134-	L-2/X-135-	L-2/X-136-	L-2/X-137-	L-2/X-138-
L-2/X-139-	L-2/X-140-	L-2/X-141-	L-2/X-142-	L-2/X-143-	L-2/X-144-
L-2/X-145-	L-2/X-146-	L-2/X-147-	L-2/X-148-	L-2/X-149-	L-2/X-150-
L-2/X-151-	L-2/X-152-	L-2/X-153-	L-2/X-154-	L-2/X-155-	L-2/X-156-
L-2/X-157-	L-2/X-158-	L-2/X-159-	L-2/X-160-	L-2/X-161-	L-2/X-162-
L-2/X-163-	L-2/X-164-	L-2/X-165-	L-2/X-166-	L-2/X-167-	L-2/X-168-
L-2/X-169-	L-2/X-170-	L-2/X-171-	L-2/X-172-		
L-2/X-173-	L-2/X-174-	L-2/X-175-	L-2/X-176-	L-2/X-177-	L-2/X-178-
L-2/X-179-	L-2/X-180-	L-2/X-181-	L-2/X-182-	L-2/X-183-	L-2/X-184-
L-2/X-185-	L-2/X-186-	L-2/X-187-	L-2/X-188-	L-2/X-189-	L-2/X-190-
L-2/X-191-	L-2/X-192-	L-2/X-193-	L-2/X-194-	L-2/X-195-	L-2/X-196-
L-2/X-197-	L-2/X-198-	L-2/X-199-	L-2/X-200-	L-2/X-201-	L-2/X-202-
L-2/X-203-	L-2/X-204-	L-2/X-205-	L-2/X-206-	L-2/X-207-	L-2/X-208-
L-2/X-209-	L-2/X-210-	L-2/X-211-	L-2/X-212-	L-2/X-213-	L-2/X-214-
L-2/X-215-	L-2/X-216-	L-2/X-217-	L-2/X-218-	L-2/X-219-	L-2/X-220-
L-2/X-221-	L-2/X-222-	L-2/X-223-	L-2/X-224-	L-2/X-225-	L-2/X-226-
L-2/X-227-	L-2/X-228-	L-2/X-229-	L-2/X-230-	L-2/X-231-	L-2/X-232-
L-2/X-233-	L-2/X-234-	L-2/X-235-	L-2/X-236-	L-2/X-237-	L-2/X-238-
L-2/X-239-	L-2/X-240-	L-2/X-241-	L-2/X-242-	L-2/X-243-	L-2/X-244-
L-2/X-245-	L-2/X-246-	L-2/X-247-	L-2/X-248-	L-2/X-249-	L-2/X-250-
L-2/X-251-	L-2/X-252-	L-2/X-253-	L-2/X-254-	L-2/X-255-	L-2/X-256-
L-2/X-257-	L-2/X-258-	L-2/X-259-	L-2/X-260-	L-2/X-261-	L-2/X-262-
L-2/X-263-	L-2/X-264-	L-2/X-265-	L-2/X-266-	L-2/X-267-	L-2/X-268-
L-2/X-269-	L-2/X-270-	L-2/X-271-	L-2/X-272-	L-2/X-273-	L-2/X-274-
L-2/X-275-	L-2/X-276-	L-2/X-277-	L-2/X-278-	L-2/X-279-	L-2/X-280-
L-2/X-281-	L-2/X-282-	L-2/X-283-	L-2/X-284-	L-2/X-285-	L-2/X-286-
L-2/X-287-	L-2/X-288-	L-2/X-289-	L-2/X-290-	L-2/X-291-	L-2/X-292-
L-2/X-293-	L-2/X-294-	L-2/X-295-	L-2/X-296-	L-2/X-297-	L-2/X-298-
L-2/X-299-	L-2/X-300-	L-2/X-301-	L-2/X-302-	L-2/X-303-	L-2/X-304-
L-2/X-305-	L-2/X-306-	L-2/X-307-	L-2/X-308-	L-2/X-309-	L-2/X-310-
L-2/X-311-	L-2/X-312-	L-2/X-313-	L-2/X-314-	L-2/X-315-	L-2/X-316-
L-2/X-317-	L-2/X-318-	L-2/X-319-	L-2/X-320-	L-2/X-321-	L-2/X-322-
L-2/X-323-	L-2/X-324-	L-2/X-325-	L-2/X-326-	L-2/X-327-	L-2/X-328-
L-2/X-329-	L-2/X-330-	L-2/X-331-	L-2/X-332-	L-2/X-333-	L-2/X-334-
L-2/X-335-	L-2/X-336-	L-2/X-337-	L-2/X-338-	L-2/X-339-	L-2/X-340-
L-2/X-341-	L-2/X-342-	L-2/X-343-	L-2/X-344-	L-2/X-345-	L-2/X-346-
L-2/X-347-	L-2/X-348-	L-2/X-349-	L-2/X-350-	L-2/X-351-	L-2/X-352-
L-2/X-353-	L-2/X-354-	L-2/X-355-	L-2/X-356-	L-2/X-357-	L-2/X-358-
L-2/X-359-	L-2/X-360-	L-2/X-361-	L-2/X-362-	L-2/X-363-	L-2/X-364-
L-2/X-365-	L-2/X-366-	L-2/X-367-	L-2/X-368-	L-2/X-369-	L-2/X-370-
L-2/X-371-	L-2/X-372-	L-2/X-373-	L-2/X-374-	L-2/X-375-	L-2/X-376-
L-2/X-377-	L-2/X-378-	L-2/X-379-	L-2/X-380-	L-2/X-381-	L-2/X-382-
L-2/X-383-	L-2/X-384-	L-2/X-385-	L-2/X-386-	L-2/X-387-	L-2/X-388-

L-2/X-389-	L-2/X-390-	L-2/X-391-	L-2/X-392-	L-2/X-393-	L-2/X-394-
L-2/X-395-	L-2/X-396-	L-2/X-397-	L-2/X-398-	L-2/X-399-	L-2/X-400-
L-2/X-401-	L-2/X-402-	L-2/X-403-	L-2/X-404-	L-2/X-405-	L-2/X-406-
L-2/X-407-	L-2/X-408-	L-2/X-409-	L-2/X-410-	L-2/X-411-	L-2/X-412-
L-2/X-413-	L-2/X-414-	L-2/X-415-	L-2/X-416-	L-2/X-417-	L-2/X-418-

L-3/X-1-	L-3/X-2-	L-3/X-3-	L-3/X-4-	L-3/X-5-	L-3/X-6-
L-3/X-7-	L-3/X-8-	L-3/X-9-	L-3/X-10-	L-3/X-11-	L-3/X-12-
L-3/X-13-	L-3/X-14-	L-3/X-15-	L-3/X-16-	L-3/X-17-	L-3/X-18-
L-3/X-19-	L-3/X-20-	L-3/X-21-	L-3/X-22-	L-3/X-23-	L-3/X-24-
L-3/X-25-	L-3/X-26-	L-3/X-27-	L-3/X-28-	L-3/X-29-	L-3/X-30-
L-3/X-31-	L-3/X-32-	L-3/X-33-	L-3/X-34-	L-3/X-35-	L-3/X-36-
L-3/X-37-	L-3/X-38-	L-3/X-39-	L-3/X-40-	L-3/X-41-	L-3/X-42-
L-3/X-43-	L-3/X-44-	L-3/X-45-	L-3/X-46-	L-3/X-47-	L-3/X-48-
L-3/X-49-	L-3/X-50-	L-3/X-51-	L-3/X-52-	L-3/X-53-	L-3/X-54-
L-3/X-55-	L-3/X-56-	L-3/X-57-	L-3/X-58-	L-3/X-59-	L-3/X-60-
L-3/X-61-	L-3/X-62-	L-3/X-63-	L-3/X-64-	L-3/X-65-	L-3/X-66-
L-3/X-67-	L-3/X-68-	L-3/X-69-	L-3/X-70-	L-3/X-71-	L-3/X-72-
L-3/X-73-	L-3/X-74-	L-3/X-75-	L-3/X-76-	L-3/X-77-	L-3/X-78-
L-3/X-79-	L-3/X-80-	L-3/X-81-	L-3/X-82-	L-3/X-83-	L-3/X-84-
L-3/X-85-	L-3/X-86-	L-3/X-87-	L-3/X-88-	L-3/X-89-	L-3/X-90-
L-3/X-91-	L-3/X-92-	L-3/X-93-	L-3/X-94-	L-3/X-95-	L-3/X-96-
L-3/X-97-	L-3/X-98-	L-3/X-99-	L-3/X-100-	L-3/X-101-	L-3/X-102-
L-3/X-103-	L-3/X-104-	L-3/X-105-	L-3/X-106-	L-3/X-107-	L-3/X-108-
L-3/X-109-	L-3/X-110-	L-3/X-111-	L-3/X-112-	L-3/X-113-	L-3/X-114-
L-3/X-115-	L-3/X-116-	L-3/X-117-	L-3/X-118-	L-3/X-119-	L-3/X-120-
L-3/X-121-	L-3/X-122-	L-3/X-123-	L-3/X-124-	L-3/X-125-	L-3/X-126-
L-3/X-127-	L-3/X-128-	L-3/X-129-	L-3/X-130-	L-3/X-131-	L-3/X-132-
L-3/X-133-	L-3/X-134-	L-3/X-135-	L-3/X-136-	L-3/X-137-	L-3/X-138-
L-3/X-139-	L-3/X-140-	L-3/X-141-	L-3/X-142-	L-3/X-143-	L-3/X-144-
L-3/X-145-	L-3/X-146-	L-3/X-147-	L-3/X-148-	L-3/X-149-	L-3/X-150-
L-3/X-151-	L-3/X-152-	L-3/X-153-	L-3/X-154-	L-3/X-155-	L-3/X-156-
L-3/X-157-	L-3/X-158-	L-3/X-159-	L-3/X-160-	L-3/X-161-	L-3/X-162-
L-3/X-163-	L-3/X-164-	L-3/X-165-	L-3/X-166-	L-3/X-167-	L-3/X-168-
L-3/X-169-	L-3/X-170-	L-3/X-171-	L-3/X-172-		
L-3/X-173-	L-3/X-174-	L-3/X-175-	L-3/X-176-	L-3/X-177-	L-3/X-178-
L-3/X-179-	L-3/X-180-	L-3/X-181-	L-3/X-182-	L-3/X-183-	L-3/X-184-
L-3/X-185-	L-3/X-186-	L-3/X-187-	L-3/X-188-	L-3/X-189-	L-3/X-190-
L-3/X-191-	L-3/X-192-	L-3/X-193-	L-3/X-194-	L-3/X-195-	L-3/X-196-
L-3/X-197-	L-3/X-198-	L-3/X-199-	L-3/X-200-	L-3/X-201-	L-3/X-202-
L-3/X-203-	L-3/X-204-	L-3/X-205-	L-3/X-206-	L-3/X-207-	L-3/X-208-
L-3/X-209-	L-3/X-210-	L-3/X-211-	L-3/X-212-	L-3/X-213-	L-3/X-214-
L-3/X-215-	L-3/X-216-	L-3/X-217-	L-3/X-218-	L-3/X-219-	L-3/X-220-
L-3/X-221-	L-3/X-222-	L-3/X-223-	L-3/X-224-	L-3/X-225-	L-3/X-226-
L-3/X-227-	L-3/X-228-	L-3/X-229-	L-3/X-230-	L-3/X-231-	L-3/X-232-
L-3/X-233-	L-3/X-234-	L-3/X-235-	L-3/X-236-	L-3/X-237-	L-3/X-238-

L-3/X-239-	L-3/X-240-	L-3/X-241-	L-3/X-242-	L-3/X-243-	L-3/X-244-
L-3/X-245-	L-3/X-246-	L-3/X-247-	L-3/X-248-	L-3/X-249-	L-3/X-250-
L-3/X-251-	L-3/X-252-	L-3/X-253-	L-3/X-254-	L-3/X-255-	L-3/X-256-
L-3/X-257-	L-3/X-258-	L-3/X-259-	L-3/X-260-	L-3/X-261-	L-3/X-262-
L-3/X-263-	L-3/X-264-	L-3/X-265-	L-3/X-266-	L-3/X-267-	L-3/X-268-
L-3/X-269-	L-3/X-270-	L-3/X-271-	L-3/X-272-	L-3/X-273-	L-3/X-274-
L-3/X-275-	L-3/X-276-	L-3/X-277-	L-3/X-278-	L-3/X-279-	L-3/X-280-
L-3/X-281-	L-3/X-282-	L-3/X-283-	L-3/X-284-	L-3/X-285-	L-3/X-286-
L-3/X-287-	L-3/X-288-	L-3/X-289-	L-3/X-290-	L-3/X-291-	L-3/X-292-
L-3/X-293-	L-3/X-294-	L-3/X-295-	L-3/X-296-	L-3/X-297-	L-3/X-298-
L-3/X-299-	L-3/X-300-	L-3/X-301-	L-3/X-302-	L-3/X-303-	L-3/X-304-
L-3/X-305-	L-3/X-306-	L-3/X-307-	L-3/X-308-	L-3/X-309-	L-3/X-310-
L-3/X-311-	L-3/X-312-	L-3/X-313-	L-3/X-314-	L-3/X-315-	L-3/X-316-
L-3/X-317-	L-3/X-318-	L-3/X-319-	L-3/X-320-	L-3/X-321-	L-3/X-322-
L-3/X-323-	L-3/X-324-	L-3/X-325-	L-3/X-326-	L-3/X-327-	L-3/X-328-
L-3/X-329-	L-3/X-330-	L-3/X-331-	L-3/X-332-	L-3/X-333-	L-3/X-334-
L-3/X-335-	L-3/X-336-	L-3/X-337-	L-3/X-338-	L-3/X-339-	L-3/X-340-
L-3/X-341-	L-3/X-342-	L-3/X-343-	L-3/X-344-	L-3/X-345-	L-3/X-346-
L-3/X-347-	L-3/X-348-	L-3/X-349-	L-3/X-350-	L-3/X-351-	L-3/X-352-
L-3/X-353-	L-3/X-354-	L-3/X-355-	L-3/X-356-	L-3/X-357-	L-3/X-358-
L-3/X-359-	L-3/X-360-	L-3/X-361-	L-3/X-362-	L-3/X-363-	L-3/X-364-
L-3/X-365-	L-3/X-366-	L-3/X-367-	L-3/X-368-	L-3/X-369-	L-3/X-370-
L-3/X-371-	L-3/X-372-	L-3/X-373-	L-3/X-374-	L-3/X-375-	L-3/X-376-
L-3/X-377-	L-3/X-378-	L-3/X-379-	L-3/X-380-	L-3/X-381-	L-3/X-382-
L-3/X-383-	L-3/X-384-	L-3/X-385-	L-3/X-386-	L-3/X-387-	L-3/X-388-
L-3/X-389-	L-3/X-390-	L-3/X-391-	L-3/X-392-	L-3/X-393-	L-3/X-394-
L-3/X-395-	L-3/X-396-	L-3/X-397-	L-3/X-398-	L-3/X-399-	L-3/X-400-
L-3/X-401-	L-3/X-402-	L-3/X-403-	L-3/X-404-	L-3/X-405-	L-3/X-406-
L-3/X-407-	L-3/X-408-	L-3/X-409-	L-3/X-410-	L-3/X-411-	L-3/X-412-
L-3/X-413-	L-3/X-414-	L-3/X-415-	L-3/X-416-	L-3/X-417-	L-3/X-418-

Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of formula I are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds of formula I above associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active

ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the compound actually administered will be determined by a physician or veterinarian, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type

described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably

orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to

the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity

Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472, which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences.*[2]

<u>Utility</u>

The compounds of this invention modulate estrogen receptor activity and accordingly, may be used for the treatment of breast cancer and to prevent the onset of osteoporosis in animals. More particularly the compounds may be used in the treatment of medical and veterinary conditions in mammals mediated by an estrogen receptor.

The compounds of the invention are particularly useful in treating breast cancer or osteoporosis mediated in one form or another by estrogen receptor activity. Accordingly, the invention also relates to pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a compound of the invention.

Additionally, the compounds of the invention may be bound to affinity resins for affinity chromatography. The compounds of the invention may be used as a tool in immunoprecipitation. The compounds may be used to identify a receptor *in vitro* for example in microscopy, electrophoresis and chromatography.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

EXAMPLE 1

The synthesis depicted in this example is illustrated in FIG. 3

Step A: Preparation of Intermediate (2a)

Compound **21** is described in Miller, J., et. al. J. Org. Chem., **50**, 2121-2123 (1985).

Step B: Preparation of a Compound of Formula I

A solution of alkyl halide **2a** (1.9 mmols), diamine **5a** (1 mmol), and diisopropylethylamine (DIPEA) (2.5 mmols) in DMF (10 mL) is maintained at reflux, and the reaction is monitored by TLC. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound depicted in FIG. 3 as being of formula I.

EXAMPLE 2

The synthesis depicted in this example is illustrated in FIG. 4

Step A: Preparation of Intermediate (26)

Compound **24** is described in Tatee, et. al.; J. Med. Chem. **22**, 1509-1517 (1979). A solution of compound **24** (1 mmol) and compound **25** (1 mmol)

in acetone (5 mL) containing K_2CO_3 is stirred and heated at reflux temperature under an inert atmosphere. The course of the reaction is followed by thin layer chromatography. When reaction occurs, the reaction solution is diluted with ethyl acetate and washed with water and with aqueous Na_2CO_3. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. Compound **26** is obtained by purification of the crude product by use of HPLC.

Step B: Preparation of Intermediate (2b)

To a solution of compound **26** (0.495 mmols) in 10 mL of dry dichloromethane at 0°C is added 0.65 mL (0.676 mmol) of 1.04 M boron tribromide in dichloromethane. After stirring for 2 h, the reaction mixture is quenched with water. After product isolation (ethyl acetate, $MgSO_4$), the product, compound **2b** is purified using chromatography.

Step C: Preparation of Compound Formula I of Figure 4

Compound **2b** is converted to the dimer of formula I of Figure 4 following the same procedure set forth above in Example 1.

EXAMPLE 3

The synthesis depicted in this example is illustrated in FIG. 5

Compound **27** is described in Tatee, et. al.; J. Med. Chem. **22**, 1509-1517 (1979). This compound is converted to dimer of formula I as depicted in FIG. 5 by following procedures set forth in Example 2.

EXAMPLE 4

The synthesis depicted in this example is illustrated in FIG. 6

Step A: Preparation of Intermediate (2d) as per Example C

Compound **29** is described in Crenshaw et. al. J. Med. Chem. **14**, 1185-1190 (1971). This compound is converted to dimer of formula I as depicted in FIG. 6 by following procedures set forth in Example 2.

EXAMPLE 5

The synthesis depicted in this example is illustrated in FIG. 7

Step A: Preparation of (methyl)-secondary diamine (5e) from primary diamine (30)

Diamine **30** (3 mmol) is dissolved in 10 mL dichloromethane under a nitrogen atmosphere. Di-tert-butyl dicarbonate (Boc_2O) (12 mmols) dissolved in 10 mL dichloromethane is added dropwise to the stirred solution and stirring is continued at room temperature. The course of the reaction is followed by TLC, and when complete, the mixture is evaporated and the precipitate collected by filtration. The precipitate is rinsed with ether.

Lithium aluminum hidride (LAH) (6 mmols) is dissolved in 200 mL tetrahydrofuran (THF) in an ice bath under nitrogen atmosphere. The compound from the above reaction (1 mmol) is dissolved in 5 mL THF and added dropwise to the LAH/THF solution. The reaction is stirred with cooloing, then warmed to room temperature, then heated to 85°C over a 30 minute period. The mixture is refluxed for 24 h, then cooled to room temperature and placed in an ice bath. Sodium sulfate decahydrate is slowly added to quench the excess LAH. The solids are removed by filtratation and rinsing with THF. The filtrate is concentrated, and purified by chromatography.

Compound **2e** is described in Jones et al., *J. Med. Chem.*, 27:1057-1066 (1984) (see FIG 15). This compound is converted to dimer of formula I as depicted in FIG. 6 by following procedures set forth in Example 2.

EXAMPLE 6

The synthesis depicted in this example is illustrated in FIG. 8

Step A: Preparation of Intermediate (3a)

Compound **2a** (5mmol), prepared as above, is added to a solution of methylamine (2g) in MeOH (40 mL). The progress of the reaction is monitored by TLC. When it is complete, the mixture is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated and the residue is chromatographed to afford the desired product.

Step B: Preparation of Formula I of FIG. 8

A solution of compound **3a** (2 mmols) and diacid **10a** (1mmol) in methylene chloride (20 mL) is prepared under argon in a flask equipped with magnetic stirrer and drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.1 mmols) while stirring at room temperature. The course of the reaction is followed by thin layer chromatography. When reaction has occurred, the reaction solution is diluted with ethyl acetate and washed with water and with aqueous Na_2CO_3. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

EXAMPLE 7

The synthesis depicted in this example is illustrated in FIG. 9

Step A: Preparation of Compound 12a

Conversion of diol **31** to dialdehyde **12a** can be accomplished following conventional techniques. See, for example March, J. Advanced Organic Chemistry 4th Edition, Reaction 0-67 (p 431)

Step B: Preparation of Intermediate (3b).

Conversion of compound **2b** to compound **3b** proceeds by contacting 1eq. of compound **2b** with approximately 5 eq. of ammonia in tetrahydrofuran. The reaction mixture is maintained at 60°C for 12 hours in a sealed vessel to provide for compound **3b**.

Step C: Preparation of Compound of Formula I

Intermediate **3b** (2 mmol) is dissolved in $CHCl_3$ (10 mL). Acetic acid (0.5 mL) is then added and the reaction is heated to reflux. Di-aldehyde **12a** (1 mmol) dissolved in CH_2Cl_2 (10 mL) is then added dropwise to the refluxing solution over 60 minutes and the reaction is refluxed for a further 60 minutes. At this point, $NaBH(OAc)_3$ is added in portions and the reaction is stirred at relux for a further 2 hours. The reaction is allowed to cool and then is quenched with aqueous NH_4Cl solution until the pH of the solution is adjusted to pH 7.0 using either 1 M HCl or 1 M NaOH. The product is extracted from this aqueous phase with EtOAc. The organic layer is dried using Na_2SO_4, the drying agent is then filtered off and the solvent removed *in vacuo* to provide the crude product. The desired material is purified from this mixture using reverse phase HPLC.

EXAMPLE 8

The synthesis depicted in this example is illustrated in FIG. 10

Step A: Preparation of Compound 13a

Conversion of diol **32** to dibromide **13a** is conventional and can be conducted in a manner described by March, J. Advanced Organic Chemistry 4th Edition, pp. 1168-69 discuss methods for stopping oxidation at level of aldehyde.

Step B: Preparation of Intermediate (3c)

Compound **2c** was converted to compound **3c** via the methods described above.

Step C: Alkylation of Compound 3c

Under a nitrogen atmosphere, 1.25 mmols of compound **3c** (4 mmols) and 2 mmols of compound **13a** are dissolved in 1mL EtOH in a sealed tube. DIPEA (5 mmols) is added, and the reaction refluxed for 12 hours. The reaction is concentrated, and the crude product purified by silica-gel chromatography.

EXAMPLE 9

The following assays are used to evaluate the multi-binding compounds of this invention.

In vitro binding assay

Using the estrogen receptor binding affinity assay described by Bergmann et al.[4] the affinity (pKi) with the estrogen receptor is determined for the multi-binding compounds of this invention.

In vivo models

There are substantial species differences in estrogen's role in bone physiology.[9] The immature rat is an excellent laboratory animal model for evaluating the effects of estrogen analogs on reproductive tissues and on bone growth. Agonistic activity is measured in ovariectomized rats, whereas antagonistic activity is determined in either ovary-intact or estrogen treated ovariectomized animals.[9]

The effects of the multibinding compounds on bone mineral density, bone histomorphometry, total serum cholesterol and uterine histology in ovariectomized rat model may be tested according to the procedures described by Ke et al.[10]

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

WHAT IS CLAIMED IS:

1. A multibinding compound and pharmaceutically acceptable salts thereof comprising 2 to 10 ligands, which may be the same or different and which are covalently attached to a linker or linkers which may be the same of different, wherein each of said ligands comprises a ligand domain capable of binding to an estrogen receptor and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

2. A multibinding compound represented by formula I:

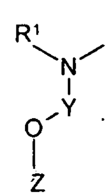
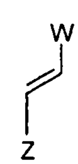
$$(L)_p(X)_q \qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20; and pharmaceutically acceptable salts thereof, and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not hexestrol in the erythro configuration.

3. The multibinding compound of Claim 2 wherein q is less than p.

4. The multibinding compound of Claim 3 wherein $p=2$ and $q=1$.

5. The multibinding compound of Claim 1 wherein said ligand is selected from the group consisting of

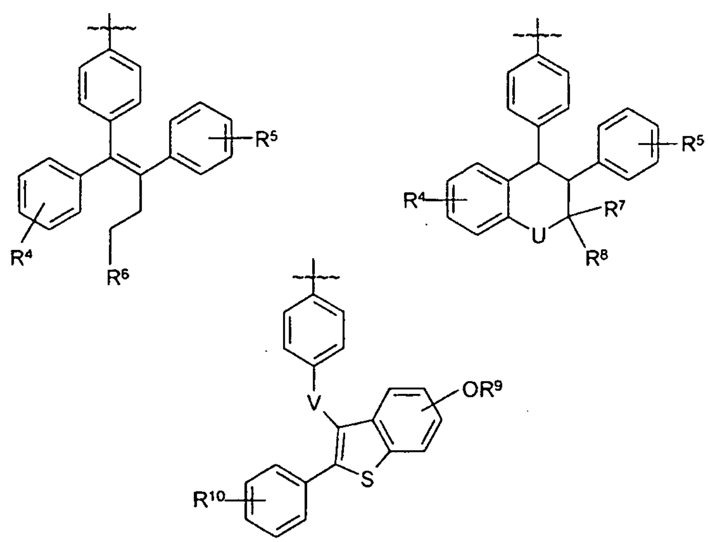
 or 

where R^1 and R^2 are selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, or are joined to form, together with the nitrogen atom to which they are pendent, a heterocyclic group;

W is selected from the group consisting of -COOH and -$COOR^3$ where R^3 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl and heterocyclic;

Y is selected from the group consisting of alkylene, alkenylene, substituted alkylene and substituted alkenylene,

Z is selected from the group consisting of:



where R^4 is selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, aryl, aryloxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxy, hydrogen, halogen, heteroaryl, heteroaryl, heterocyclic, -$OP(O)(OH)_2$, and -OSO_3H;

R^5 is selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^6 is selected from the group consisting of hydrogen, hydroxyl, halogen and cyano;

R^7, R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^{10} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

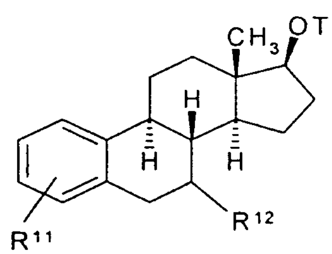
U is selected from the group consisting of methylene, ethylene, -O- and -S-; and

V is selected from the group consisting of a covalent bond, -C(O)- and -C(S)-;

with the proviso that one of R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} links the ligand to a linker;

and pharmaceutically acceptable salts thereof.

6. The multibinding compound of Claim 1 wherein the ligand has the formula:



where R^{11} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

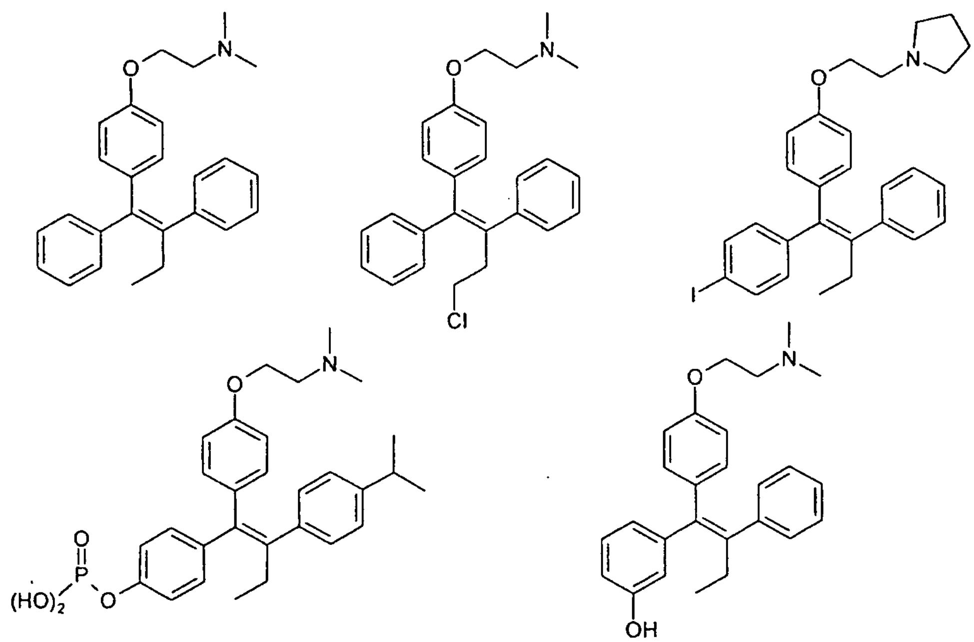
R^{12} is selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

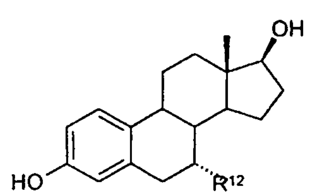
T is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, cycloalkyl, substituted cycloalkyl, heteroaryl and heterocyclic

with the proviso that at least one of R^{11}, R^{12} and T links the ligand to a linker;

and pharmaceutically acceptable salts thereof.

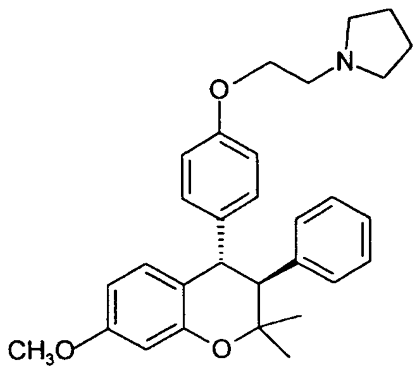
7. A multibinding compound according to Claim 1 wherein the ligand is selected from ligands having the formula:

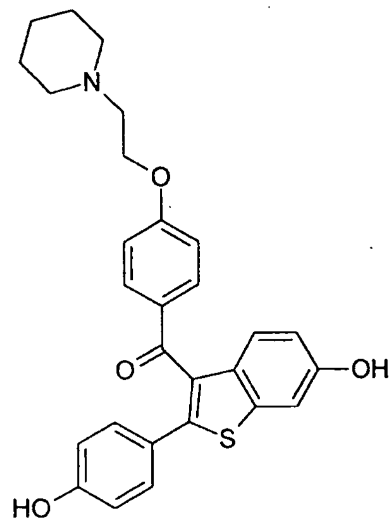


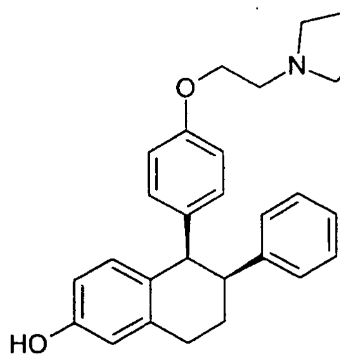


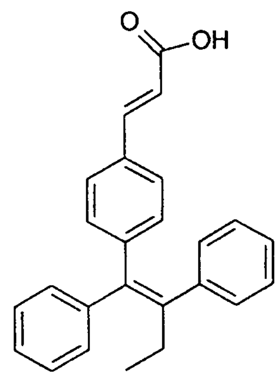
R^{12} = $-(CH_2)_{10}C(O)N(CH_3)CH_2CH_2CH_2CH_3$

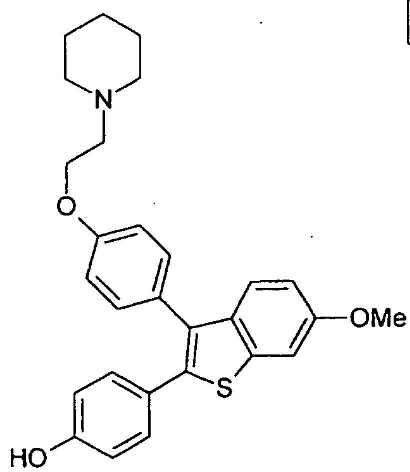
or $-(CH_2)_9S(O)(CH_2)_3CF_2CH_3$


N
O
CH3O
O


N
O
O
OH
S
HO


N
O
HO


O
OH


N
O
OMe
S
HO

wherein linkage to a linker occurs at any atom of the ligand capable of covalent attachment to the linker via conventional organic synthetic techniques as illustrated below.

8. The multibinding compound according to Claim 1 or Claim 2 wherein the compound is dimeric.

9. The multibinding compound according to Claim 8 wherein the dimeric compound is heterodimeric.

10. The multibinding compound according to Claim 1 or Claim 2 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

11. The multibinding compound according to Claim 10 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

12. The multibinding compound according to Claim 11 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

13. The multibinding compound according to Claim 12 wherein the linkers are selected to have different linker lengths ranging from about 3 to 40Å.

14. The multibinding compound according to Claim 1 or 2 wherein the linker is represented by the formula:

$$-X'-Z-(Y'-Z)_m-Y''-Z-X'-$$

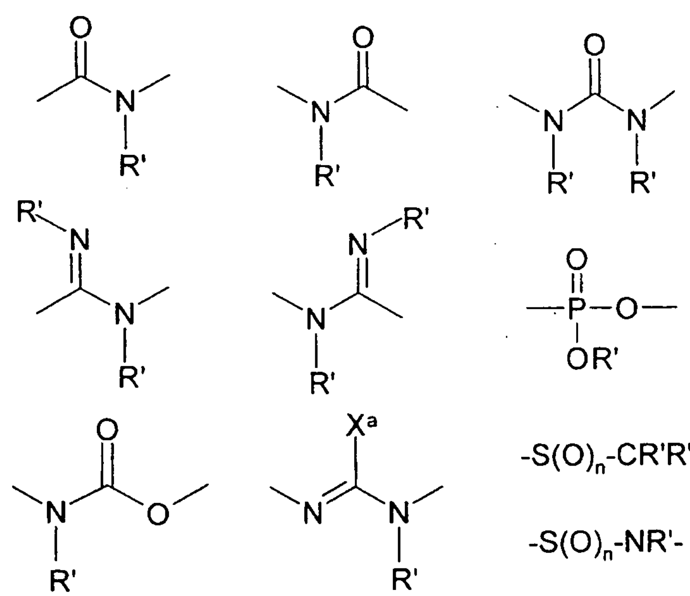
in which:

m is an integer of from 0 to 20;

X′ at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y′ and Y″ at each separate occurrence are selected from the group consisting of :



-S(O)$_n$-CR'R"-

-S(O)$_n$-NR'-

-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted

cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

15. The multibinding compound according to Claim 8 wherein the dimeric compound is homodimeric.

16. The multibinding compound according to Claim 15 wherein the two macromolecular ligands are linked to the homodimeric compound at the same point of the ligand.

17. The multibinding compound according to Claim 16 wherein the two macromolecular ligands are linked to the homodimeric compound at different points on the ligand.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound or a pharmaceutically acceptable salt thereof comprising 2 to 10 ligands, which may be the same or different and which are covalently attached to a linker or linkers which may be the same of different, wherein each of said ligands comprises a ligand domain capable of binding to an estrogen receptor and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

19. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound represented by formula I:

$$(L)_p(X)_q \qquad \mathrm{I}$$

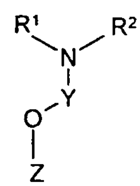
wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is independently a linker; *p* is an integer of from 2 to 10; *q* is an integer of from 1 to 20; and

pharmaceutically acceptable salts thereof, and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not hexestrol in the erythro configuration.

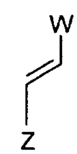
20. The pharmaceutical composition of Claim 19 wherein q is less than p.

21. The pharmaceutical composition of Claim 20 wherein $p=2$ and $q=1$.

22. The pharmaceutical composition of Claim 19 or Claim 20 wherein said ligand is selected from the group consisting of



or

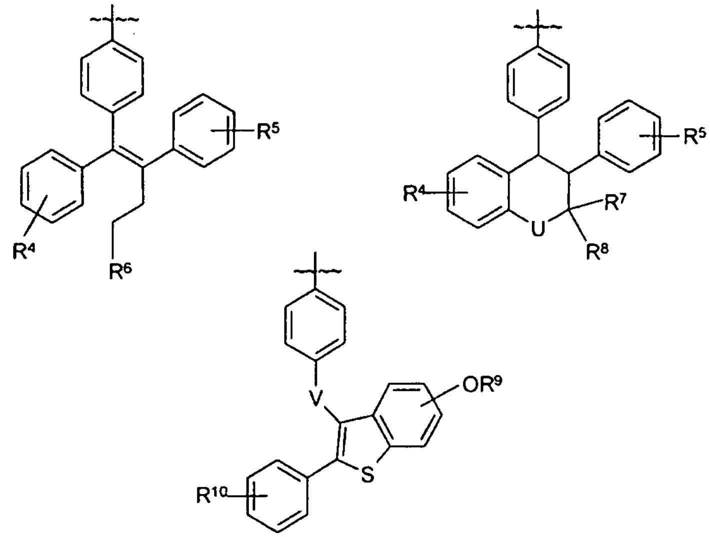


where R^1 and R^2 are selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, or are joined to form, together with the nitrogen atom to which they are pendent, a heterocyclic group;

W is selected from the group consisting of -COOH and -COOR^3 where R^3 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl and heterocyclic;

Y is selected from the group consisting of alkylene, alkenylene, substituted alkylene and substituted alkenylene,

Z is selected from the group consisting of:



where R^4 is selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, aryl, aryloxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxy, hydrogen, halogen, heteroaryl, heteroaryl, heterocyclic, $-OP(O)(OH)_2$ and $-OSO_3H$;

R^5 is selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^6 is selected from the group consisting of hydrogen, hydroxyl, halogen and cyano;

R^7, R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^{10} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

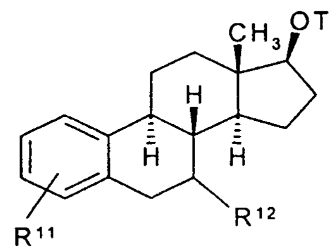
U is selected from the group consisting of methylene, ethylene, -O- and -S-; and

V is selected from the group consisting of a covalent bond, -C(O)- and -C(S)-;

with the proviso that one of R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} links the ligand to a linker;

and pharmaceutically acceptable salts thereof.

23. The pharmaceutical composition according to Claim 19 or Claim 20 wherein the ligand has the formula:



where R^{11} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

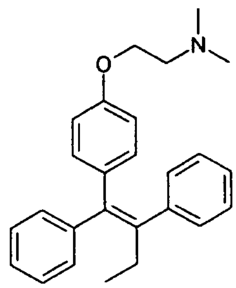
R^{12} is selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

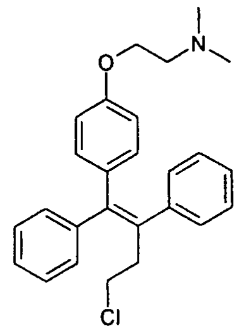
T is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, cycloalkyl, substituted cycloalkyl, heteroaryl and heterocyclic

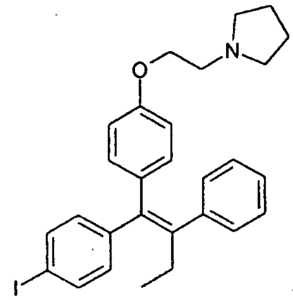
with the proviso that at least one of R^{11}, R^{12} and T links the ligand to a linker;

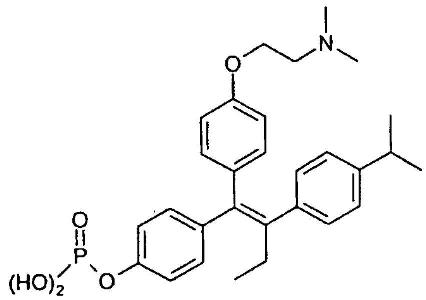
and pharmaceutically acceptable salts thereof.

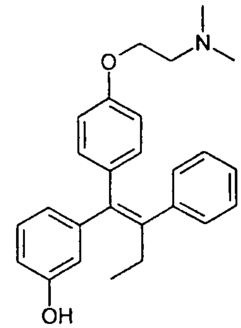
24. The pharmaceutical composition according to Claim 1 wherein the ligand is selected from ligands having the formula:

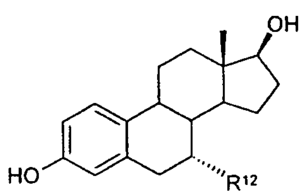






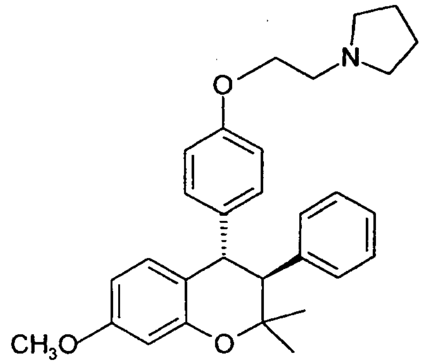


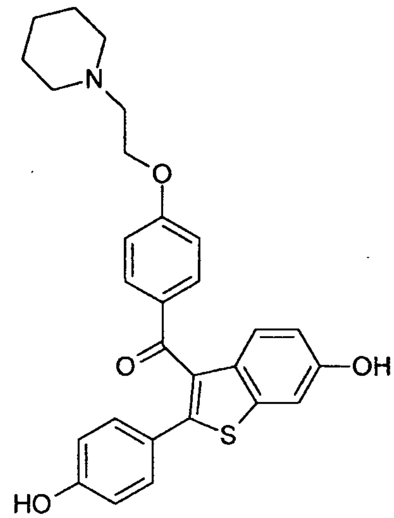


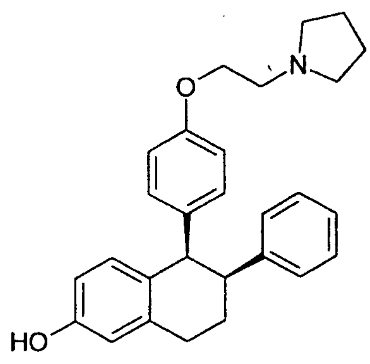


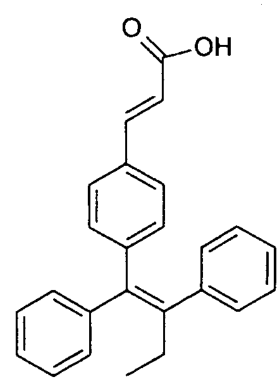
R^{12} = $-(CH_2)_{10}C(O)N(CH_3)CH_2CH_2CH_2CH_3$

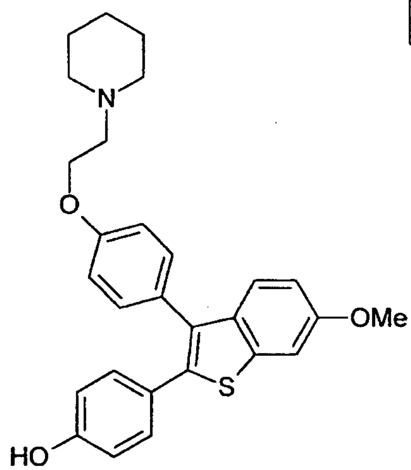
or $-(CH_2)_9S(O)(CH_2)_3CF_2CH_3$











wherein linkage to a linker occurs at any atom of the ligand capable of covalent attachment to the linker via conventional organic synthetic techniques as illustrated below.

25. The pharmaceutical composition according to Claim 19 or Claim 20 wherein the compound is dimeric.

26. The pharmaceutical composition according to Claim 25 wherein the dimeric compound is heterodimeric.

27. The pharmaceutical composition according to Claim 19 or Claim 20 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

28. The pharmaceutical composition according to Claim 27 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

29. The pharmaceutical composition according to Claim 28 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

30. The pharmaceutical composition according to Claim 29 wherein the linkers are selected to have different linker lengths ranging from about 3 to 40Å.

31. The pharmaceutical composition according to Claim 19 or 20 wherein the linker is represented by the formula:

$$-X'-Z-(Y'-Z)_m-Y''-Z-X'-$$

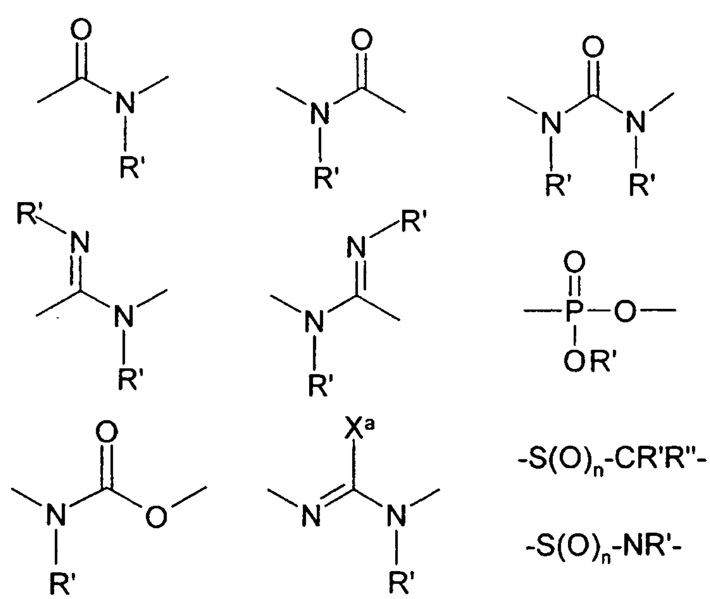
in which:

m is an integer of from 0 to 20;

X′ at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y′ and Y″ at each separate occurrence are selected from the group consisting of :



-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

32. The pharmaceutical composition according to Claim 25 wherein the dimeric compound is homodimeric.

33. The pharmaceutical composition according to Claim 32 wherein the two macromolecular ligands are linked to the homodimeric compound at the same point of the ligand.

34. The pharmaceutical composition according to Claim 33 wherein the two macromolecular ligands are linked to the homodimeric compound at different points on the ligand.

35. A method for treating breast cancer mediated by estrogen receptors in a mammal which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound, or a pharmaceutically acceptable salt thereof, comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, at least two of said ligands comprising a ligand domain capable of binding to an estrogen receptor and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

36. A method for treating breast cancer mediated by estrogen receptors in a mammal mediated which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a

pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

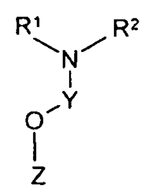
$$(L)_p(X)_q \qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and pharmaceutically acceptable salts thereof and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

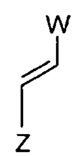
37. The method of Claim 36 wherein q is less than p.

38. The method of Claim 37 wherein $p=2$ and $q=1$.

39. The method of Claim 35 or 36 wherein said ligand is selected from the group consisting of



or



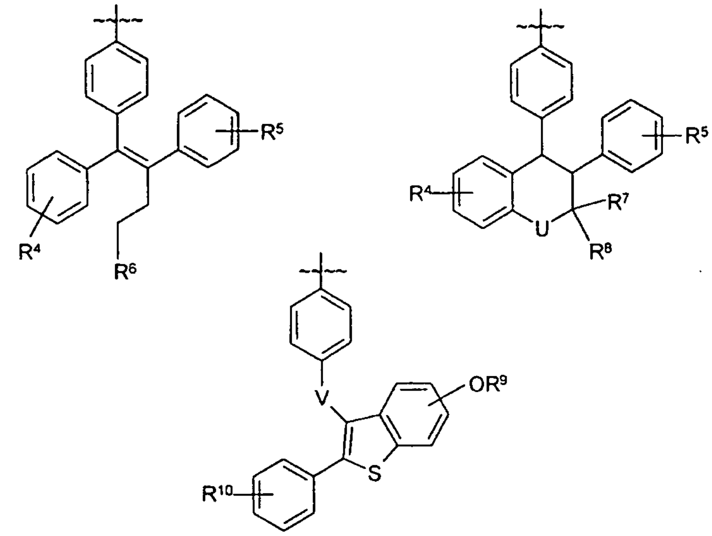
where R^1 and R^2 are selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, or are joined to form, together with the nitrogen atom to which they are pendent, a heterocyclic group;

W is selected from the group consisting of -COOH and -$COOR^3$ where R^3 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl and heterocyclic;

Y is selected from the group consisting of alkylene, alkenylene, substituted

alkylene and substituted alkenylene,

Z is selected from the group consisting of:



where R^4 is selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, aryl, aryloxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxy, hydrogen, halogen, heteroaryl, heteroaryl, heterocyclic, $-OP(O)(OH)_2$, and $-OSO_3H$;

R^5 is selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^6 is selected from the group consisting of hydrogen, hydroxyl, halogen and cyano;

R^7, R^8 and R^9 are independently selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R^{10} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

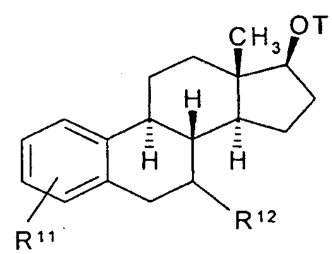
U is selected from the group consisting of methylene, ethylene, -O- and -S-; and

V is selected from the group consisting of a covalent bond, -C(O)- and -C(S)-;

with the proviso that one of R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} links the ligand to a linker;

and pharmaceutically acceptable salts thereof.

40. The method of Claim 35 or 36 wherein the ligand has the formula:



where R^{11} is selected from the group consisting of hydrogen, hydroxyl, alkoxy and substituted alkoxy;

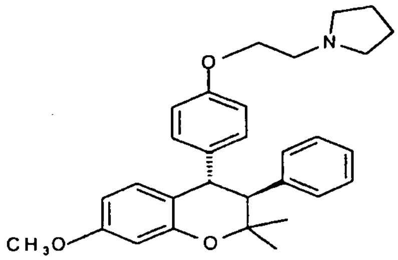
R^{12} is selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

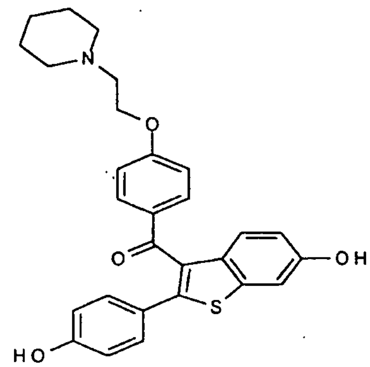
T is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, cycloalkyl, substituted cycloalkyl, heteroaryl and heterocyclic

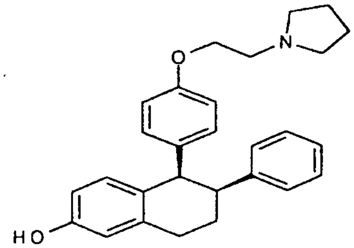
with the proviso that at least one of R^{11}, R^{12} and T links the ligand to a linker;

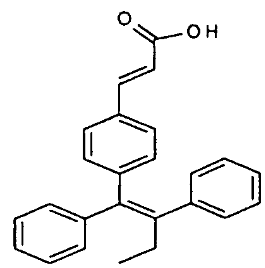
and pharmaceutically acceptable salts thereof.

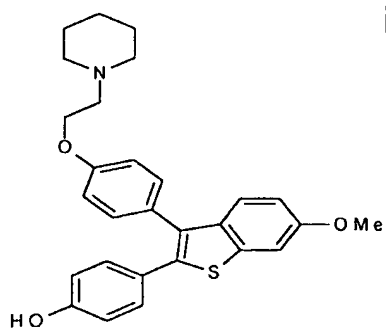
41. The method of Claim 35 or 36 wherein the ligand is selected from ligands having the formula:

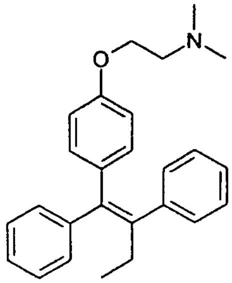


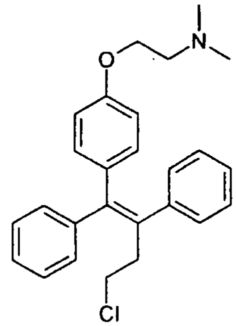


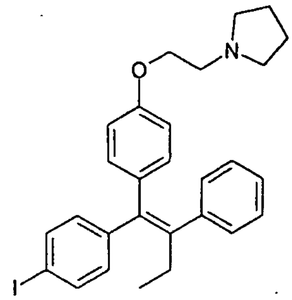


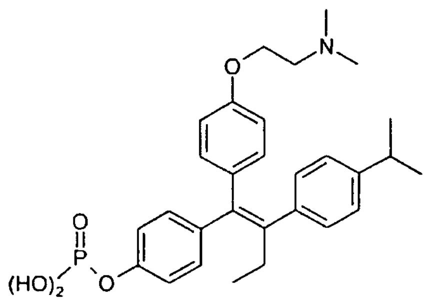


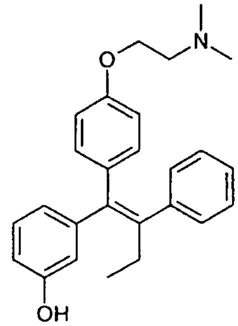


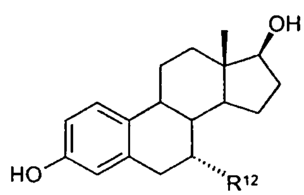












R^{12} = $-(CH_2)_{10}C(O)N(CH_3)CH_2CH_2CH_2CH_3$

or $-(CH_2)_9S(O)(CH_2)_3CF_2CH_3$

wherein linkage to a linker occurs at any atom of the ligand capable of covalent attachment to the linker via conventional organic synthetic techniques as illustrated below.

42. The method of Claim 35 or Claim 36 wherein the compound is dimeric.

43. The method according to Claim 42 wherein the dimeric compound is heterodimeric.

44. The method of Claim 35 or Claim 36 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

45. The method of Claim 44 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

46. The method of Claim 45 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

47. The method of Claim 46 wherein the linkers are selected to have different linker lengths ranging from about 3 to 40Å.

48. The method of Claims 35 or 36 wherein the linker is represented by the formula::

$$-X'-Z-(Y'-Z)_m-Y''-Z-X'-$$

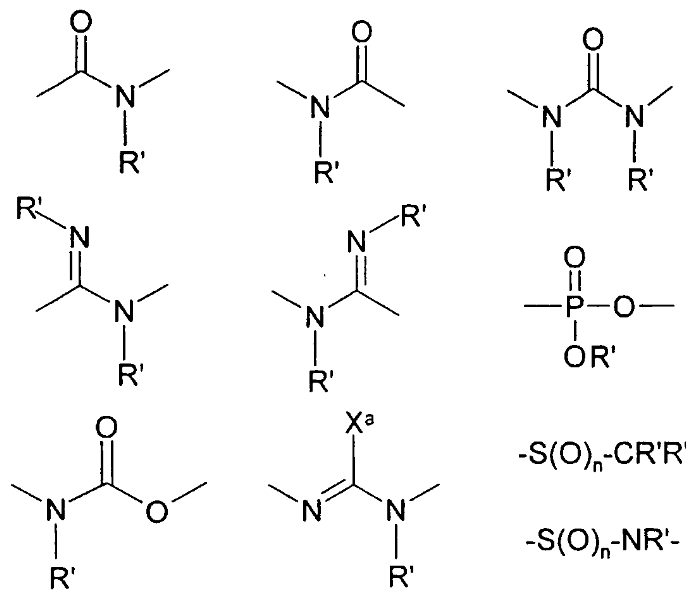
in which:

m is an integer of from 0 to 20;

X′ at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y′ and Y″ at each separate occurrence are selected from the group consisting of :



$-S(O)_n-CR'R''-$

$-S(O)_n-NR'-$

-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

49. The method according to Claim 42 wherein the dimeric compound is homodimeric.

50. The method according to Claim 49 wherein the two macromolecular ligands are linked to the homodimeric compound at the same point of the ligand.

51. The method according to Claim 50 wherein the two macromolecular ligands are linked to the homodimeric compound at different points on the ligand.

52. A method for identifying multimeric ligand compounds possessing multibinding properties against estrogen receptors which method comprises:

(a) identifying an estrogen receptor ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties against estrogen receptors.

53. A method for identifying multimeric ligand compounds possessing multibinding properties against estrogen receptors which method comprises:

(a) identifying a library of estrogen receptor ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the

complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties against estrogen receptors.

54. The method of Claim 52 or Claim 53 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

55. The method of Claim 54 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

56. The method of Claim 55 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

57. The method of Claim 56 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

58. The method of Claim 52 or Claim 53 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

59. The method of Claim 58 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

60. The method of Claim 52 or Claim 53 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry,

acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

61. The method of Claim 60 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

62. The method according to Claim 61 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

63. The method of Claim 52 or Claim 53 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

64. The method of Claim 63 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, phosphates, phosphonates and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

65. The method of Claim 52 or Claim 53 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

66. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying an estrogen receptor ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

67. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of estrogen receptor ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

68. The library of Claim 66 or Claim 67 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

69. The library of Claim 68 wherein the multimeric ligand compound library are dimeric.

70. The library of Claim 69 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

71. The library of Claim 70 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

72. The library of Claim 66 or Claim 67 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

73. The library of Claim 72 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

74. The library of Claim 66 or Claim 67 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

75. The library of Claim 74 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

76. The library according to Claim 75 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

77. The library of Claim 66 or Claim 67 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

78. The library of Claim 77 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides,

carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, phosphates, phosphonates and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

79. The library of Claim 66 or Claim 67 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

80. A method for identifying multimeric ligand compounds possessing multibinding properties against estrogen receptors which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target an estrogen receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties against estrogen receptor;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting

multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

81. The method of claim 80 wherein steps (e) and (f) are repeated 2 to 50 times.

82. The method of claim 81 wherein steps (e) and (f) are repeated 5 to 50 times.

83. A method for treating osteoporosis mediated by estrogen receptors in a mammal which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and pharmaceutically acceptable salts thereof and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

84. The method of claim 83 wherein q is less than p.

85. A method for treating atherosclerosis mediated by estrogen receptors in a mammal which method comprises administering to said mammal

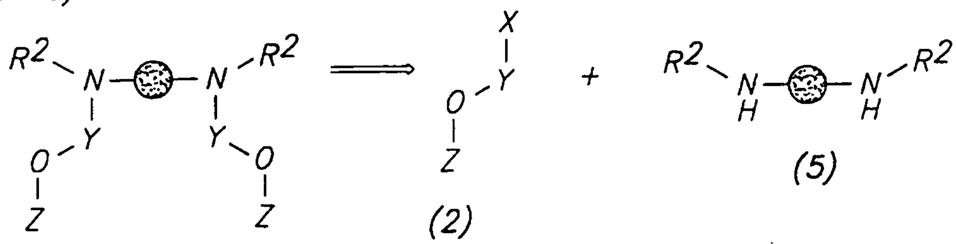
an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad \mathrm{I}$$

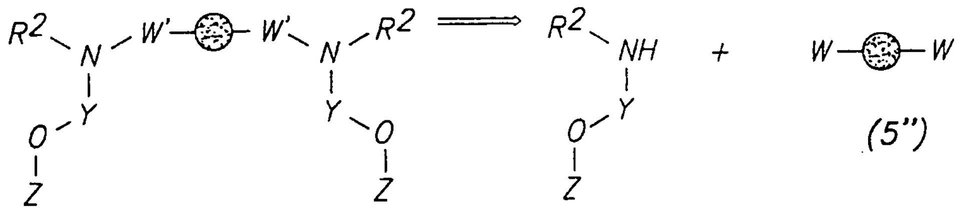
wherein each L is independently selected from ligands comprising a ligand domain capable of binding to an estrogen receptor; X is a linker; *p* is an integer of from 2 to 10; *q* is an integer of from 1 to 20 and pharmaceutically acceptable salts thereof and with the proviso that when said multibinding compound is a homodimer, then said 2 ligands are not based on estrogen hexestrol.

86. The method of claim 85 wherein *q* is less than *p*.

(SERMs)

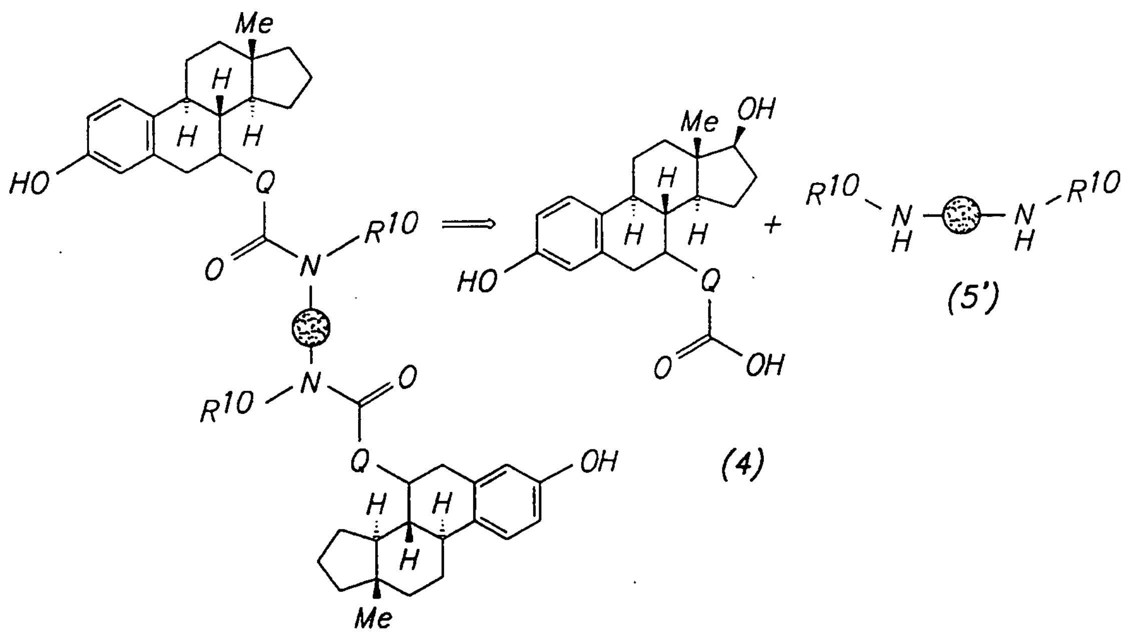


FORMULA 1a (2) (5)



FORMULA 1a (3) (5")

(STEROIDAL)



FORMULA 1b (4) (5')

FIG. 1

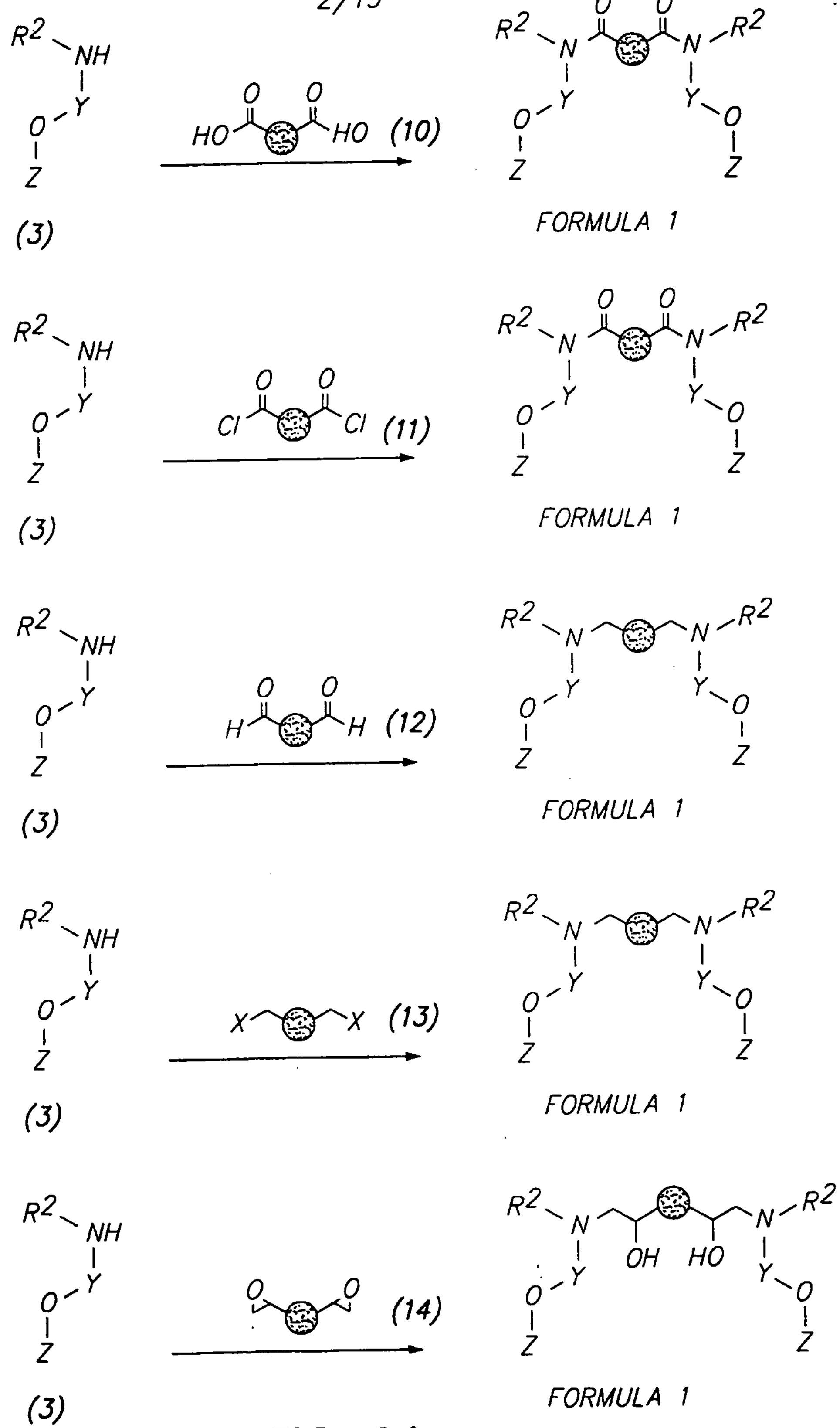
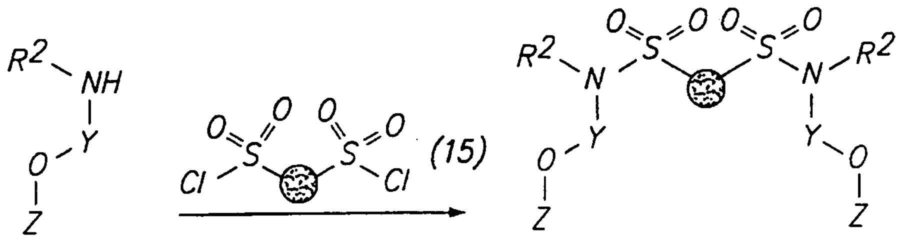
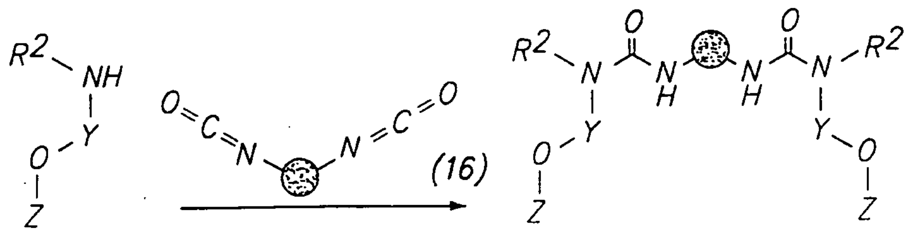

(3)
(10)
FORMULA 1
(3)
(11)
FORMULA 1
(3)
(12)
FORMULA 1
(3)
(13)
FORMULA 1
(3)
(14)
FORMULA 1

FIG. 2A

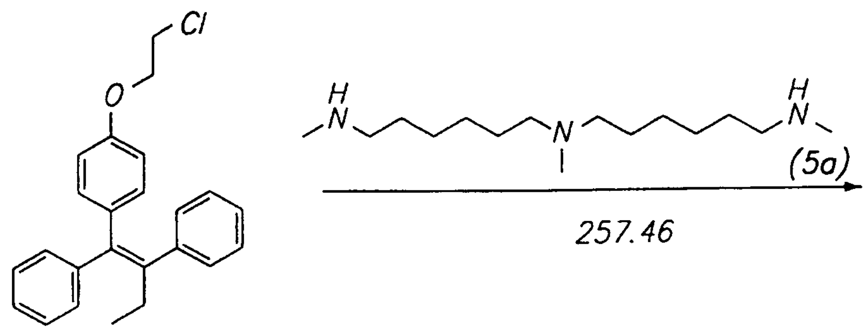


(3) FORMULA 1

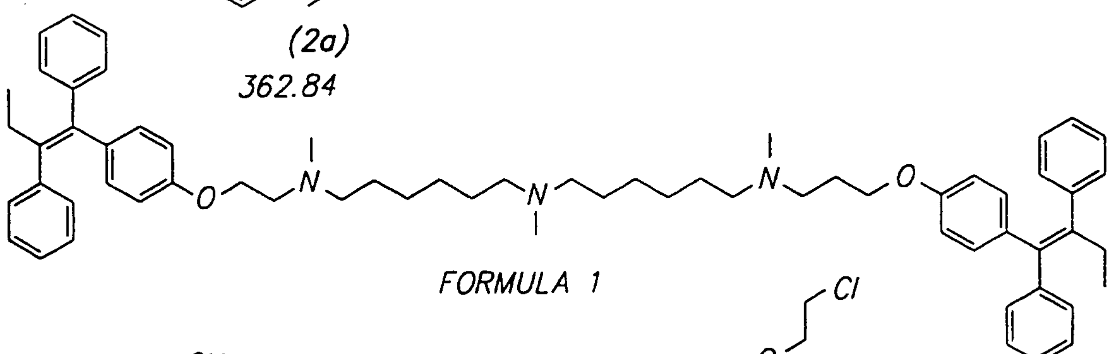


(3) FORMULA 1

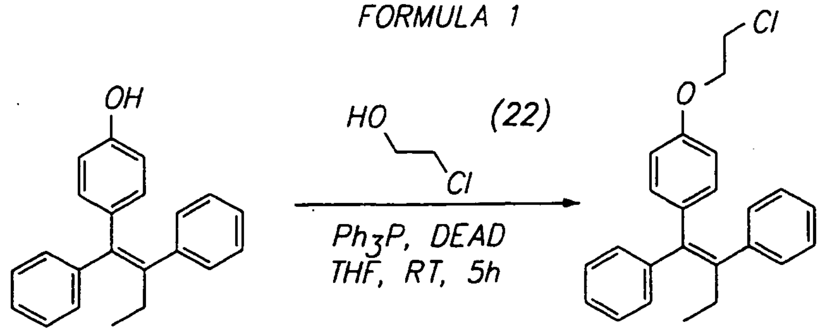
FIG. 2B



(2a)
362.84



FORMULA 1



(21) (2a)

FIG. 3

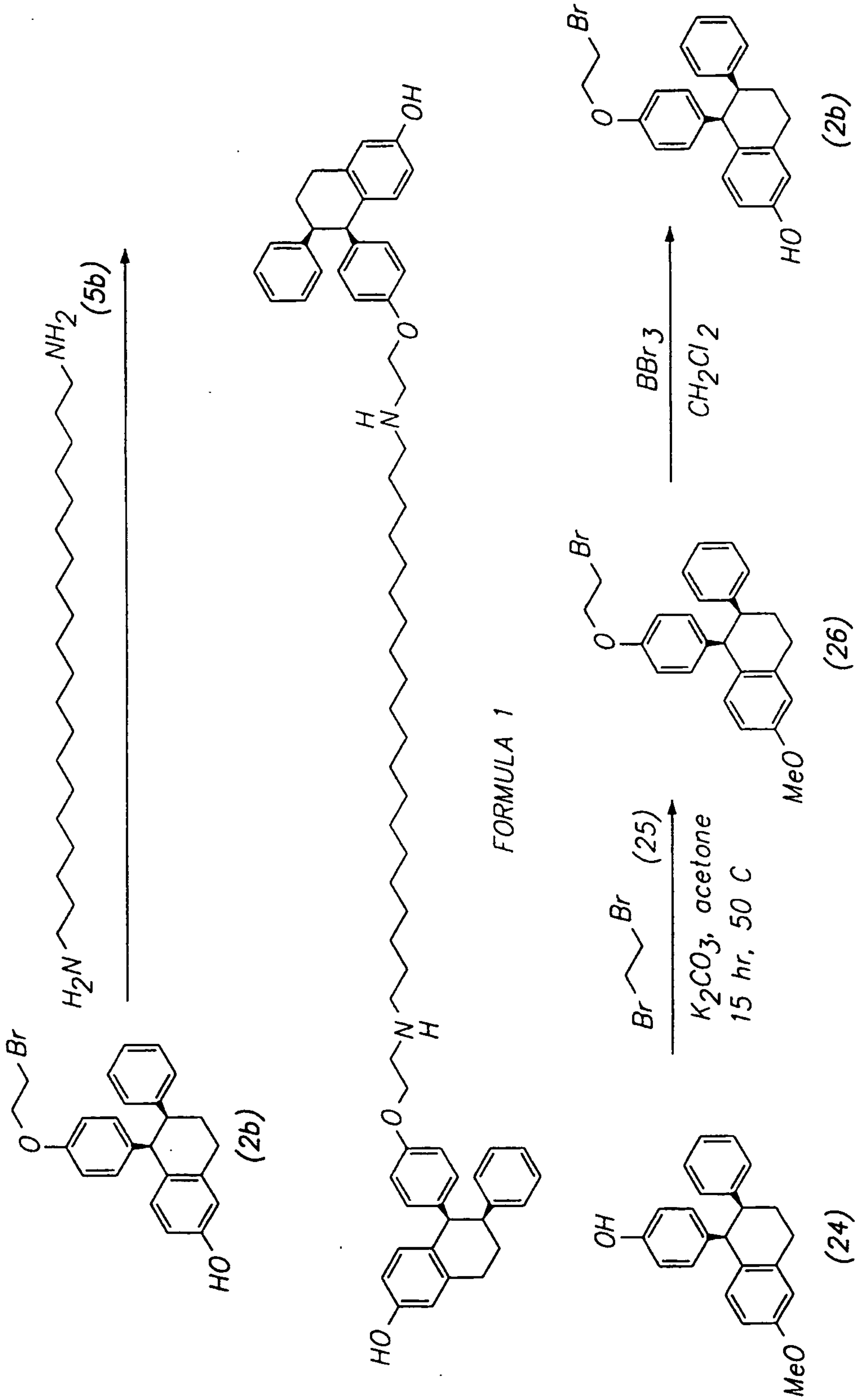

(2b)
H_2N
NH_2
(5b)
FORMULA 1
(24)
Br
Br
(25)
K_2CO_3, acetone
15 hr, 50 C
(26)
BBr_3
CH_2Cl_2
(2b)

FIG. 4

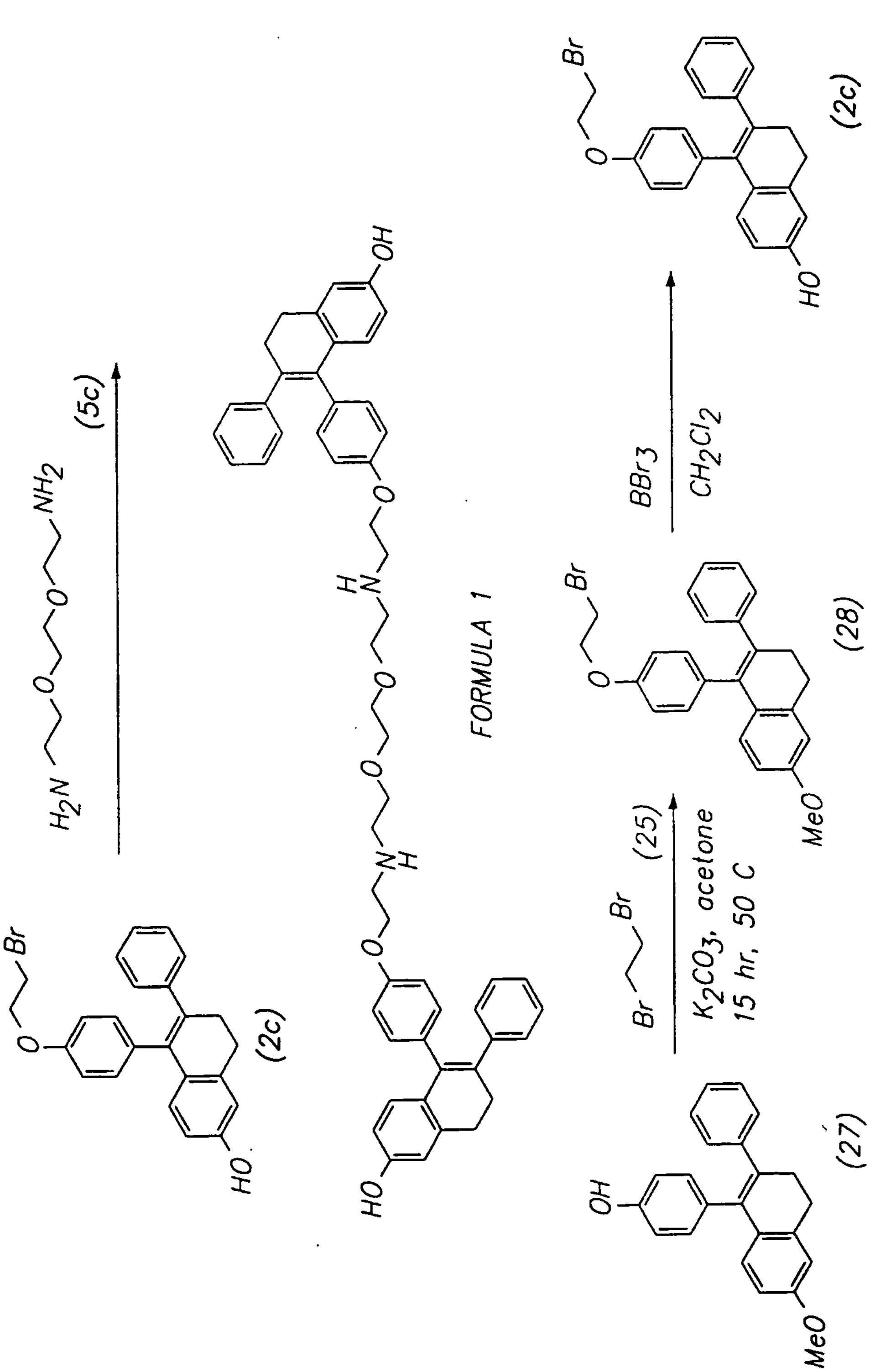

(27)
MeO
OH
Br
Br
(25)
K_2CO_3, acetone
15 hr, 50 C
(28)
MeO
Br
BBr_3
CH_2Cl_2
(2c)
HO
Br
(2c)
HO
Br
H_2N
NH_2
(5c)
HO
OH
FORMULA 1

FIG. 5

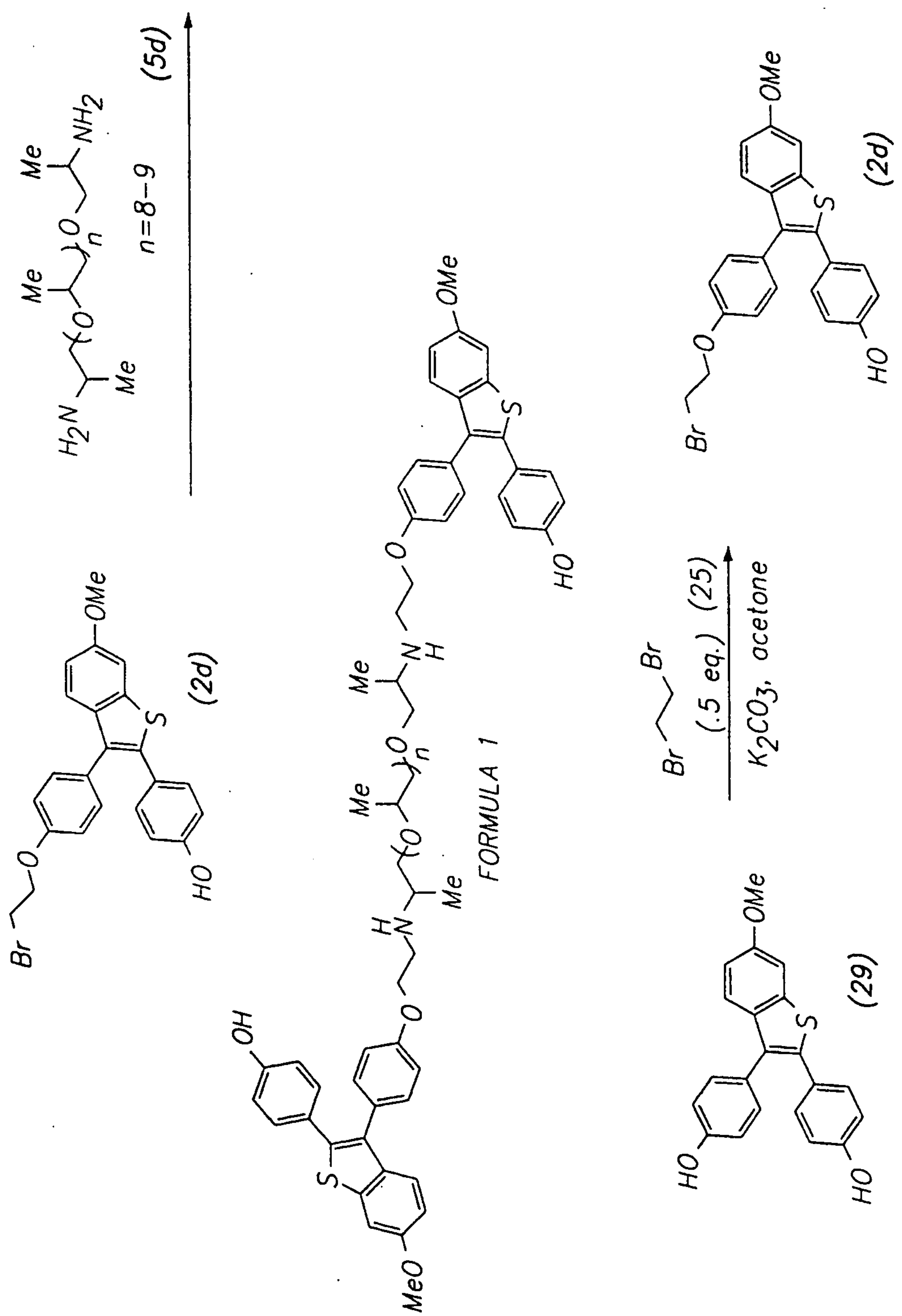


FIG. 6

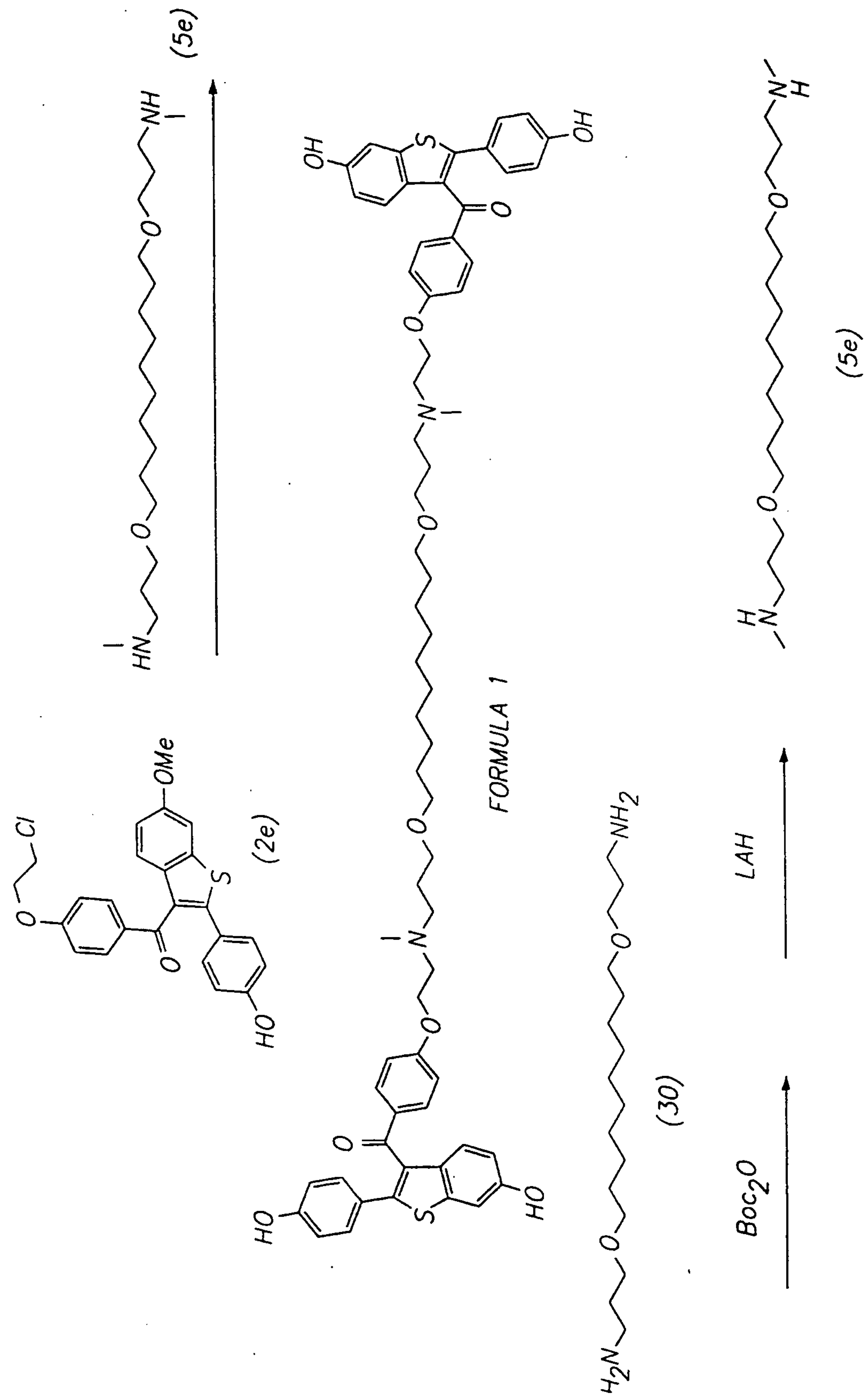


FIG. 7

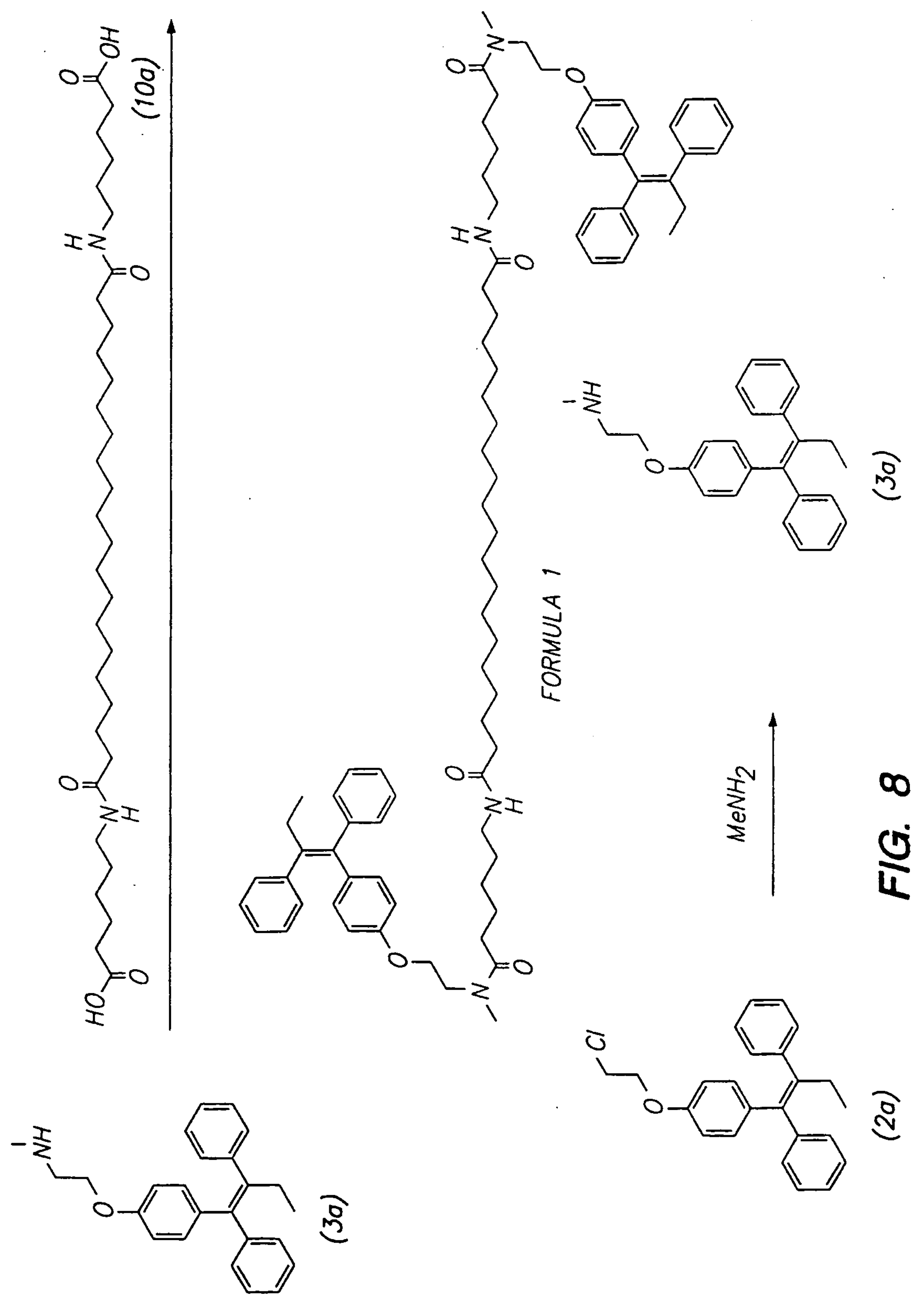
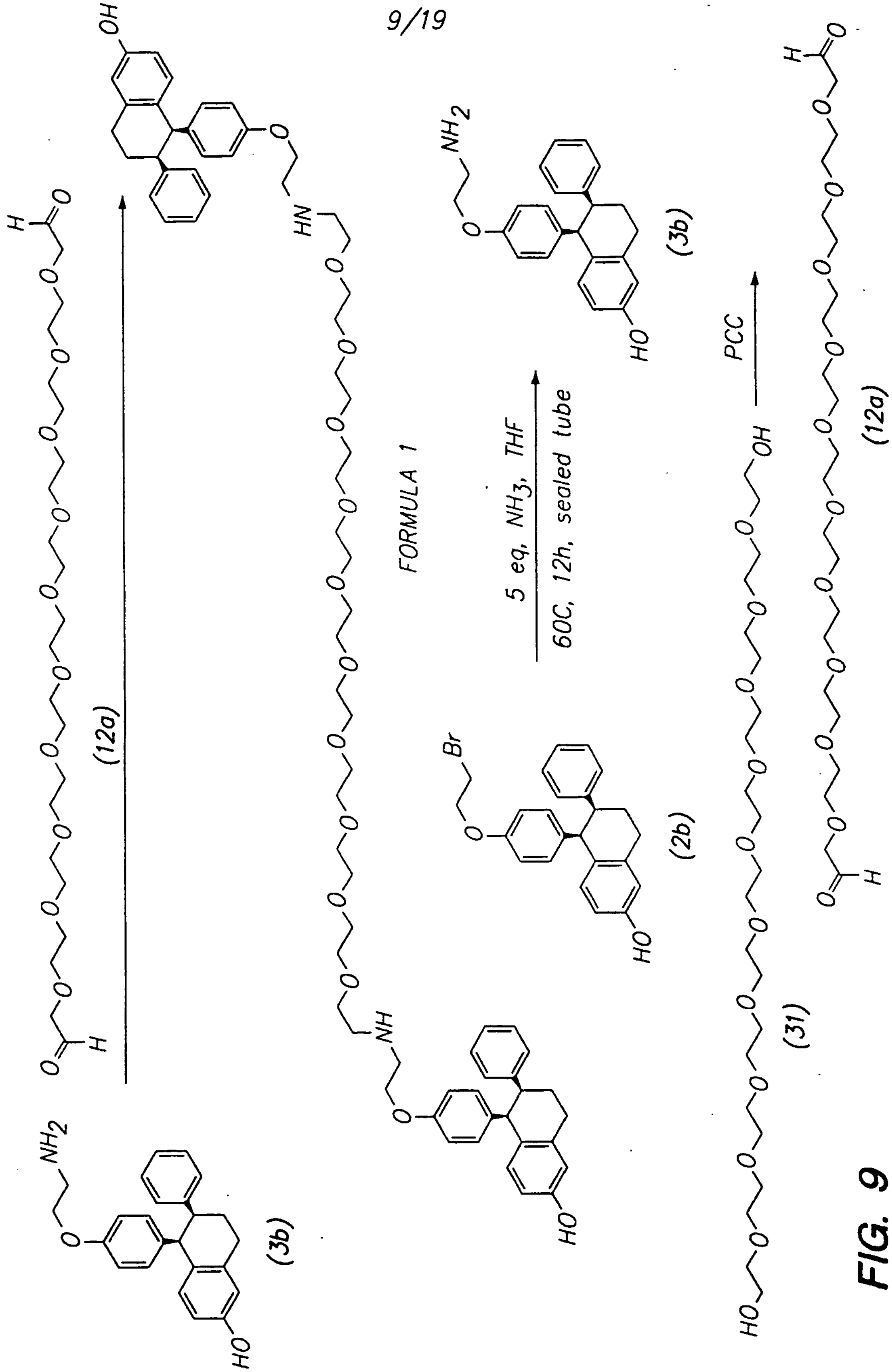


FIG. 8



(3b)
(12a)
(3b)
FORMULA 1
(2b)
5 eq, NH_3, THF
60C, 12h, sealed tube
(3b)
(31)
PCC
(12a)

FIG. 9

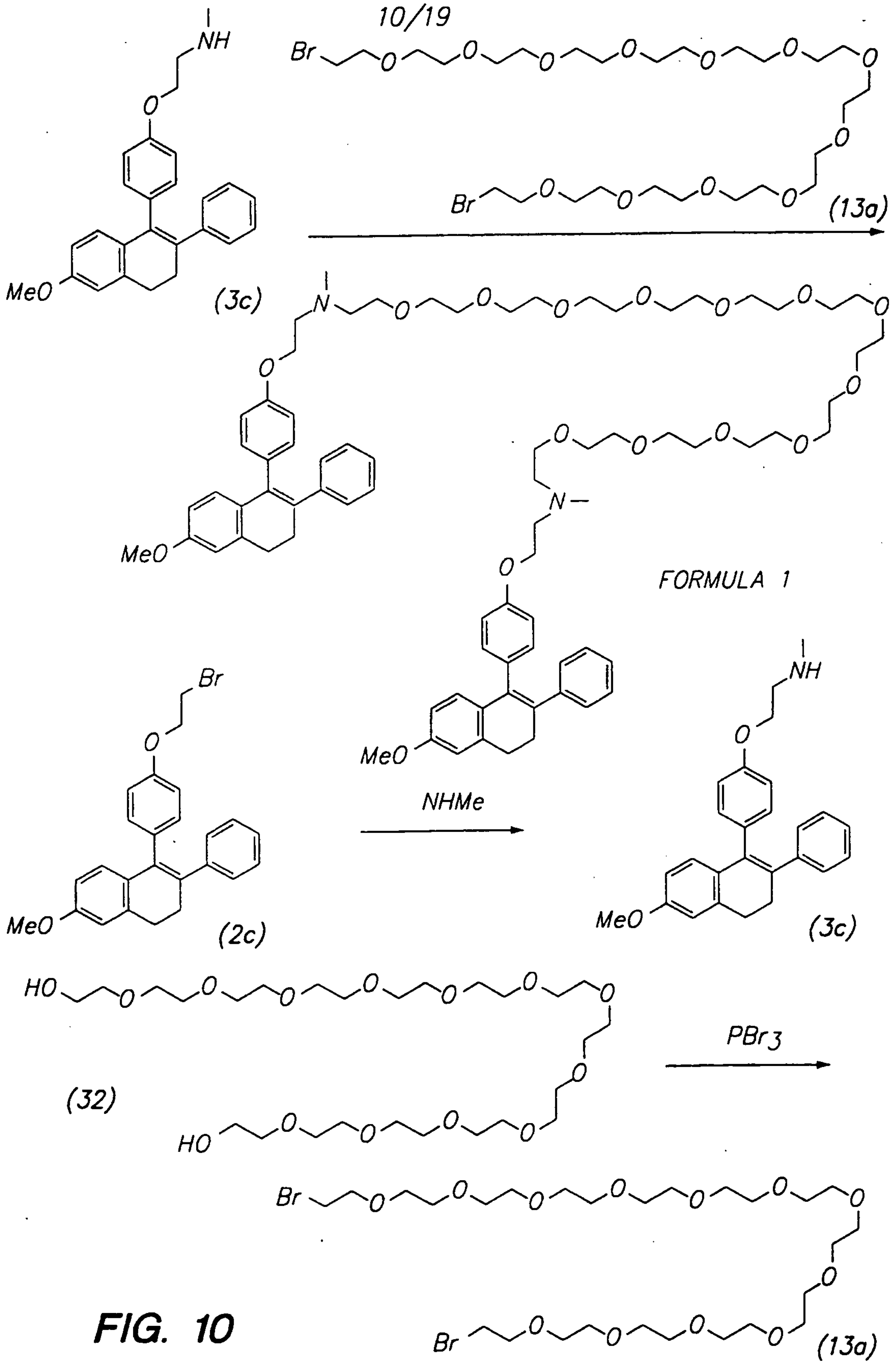


FIG. 10

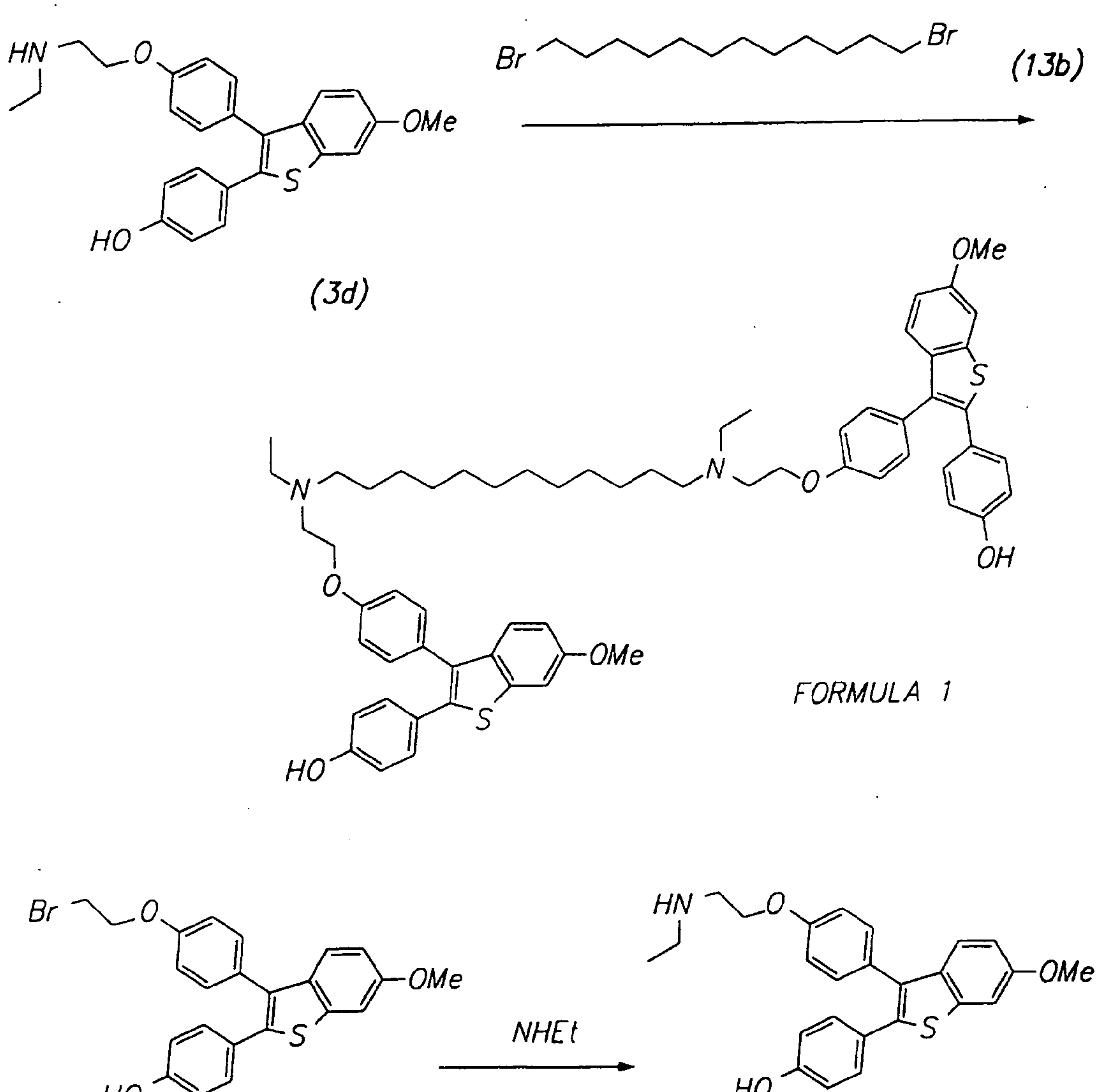


FIG. 11

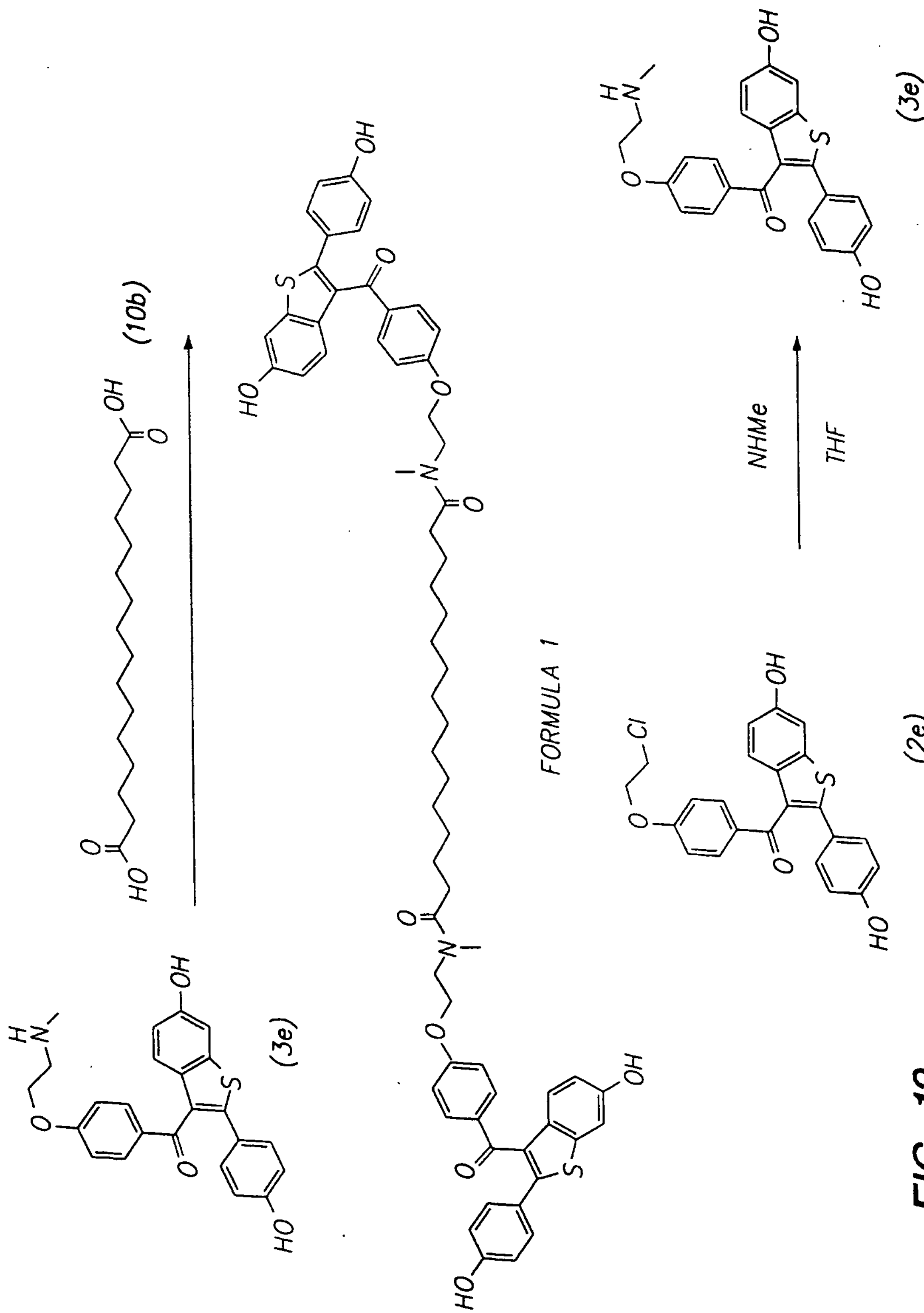
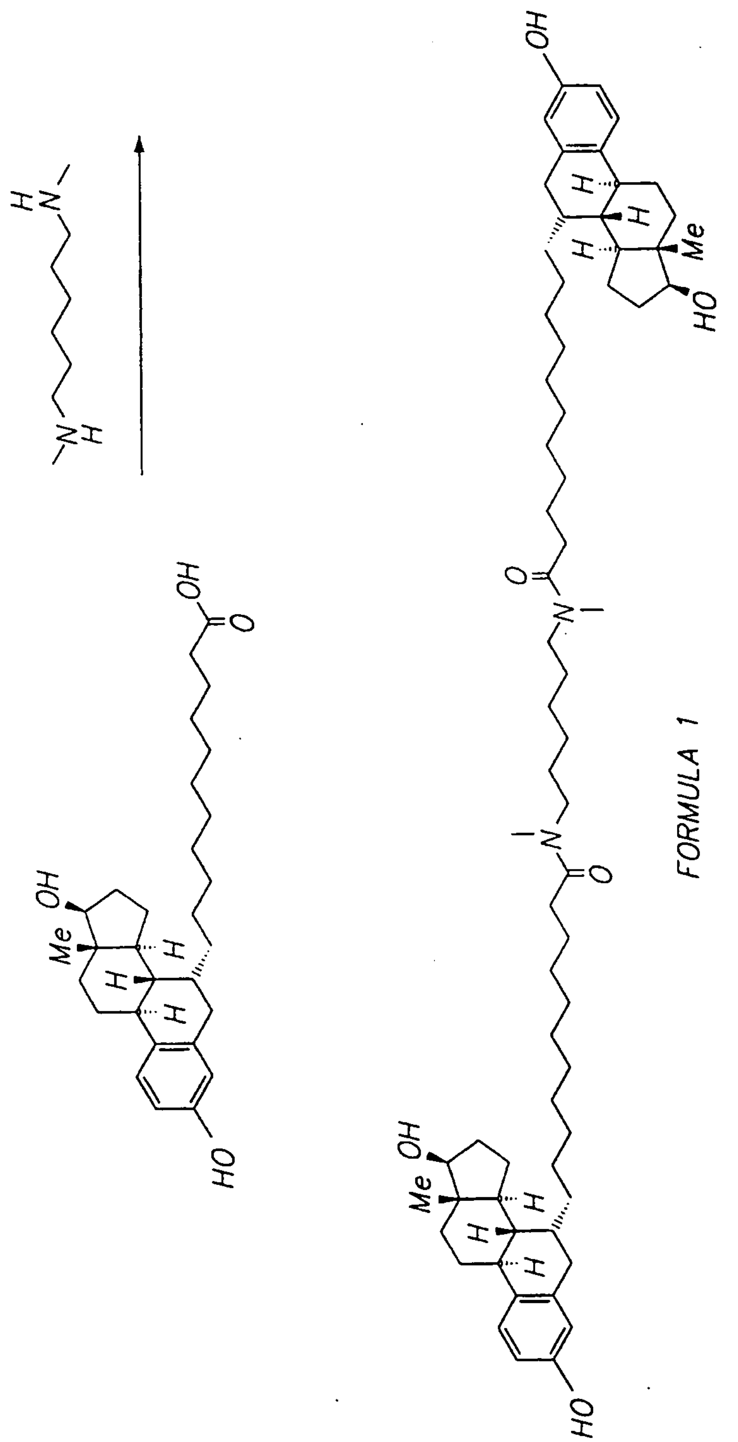
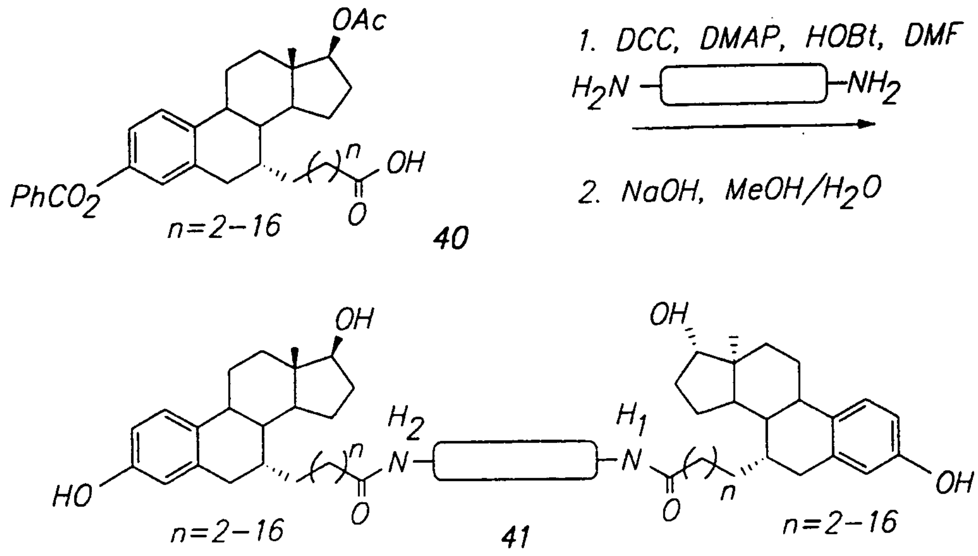


FIG. 12



FORMULA 1

FIG. 13


OAc
1. DCC, DMAP, HOBt, DMF
H_2N — — NH_2
2. NaOH, MeOH/H_2O
$PhCO_2$
n=2-16
OH
40
OH
HO
n=2-16
41
n=2-16
OH

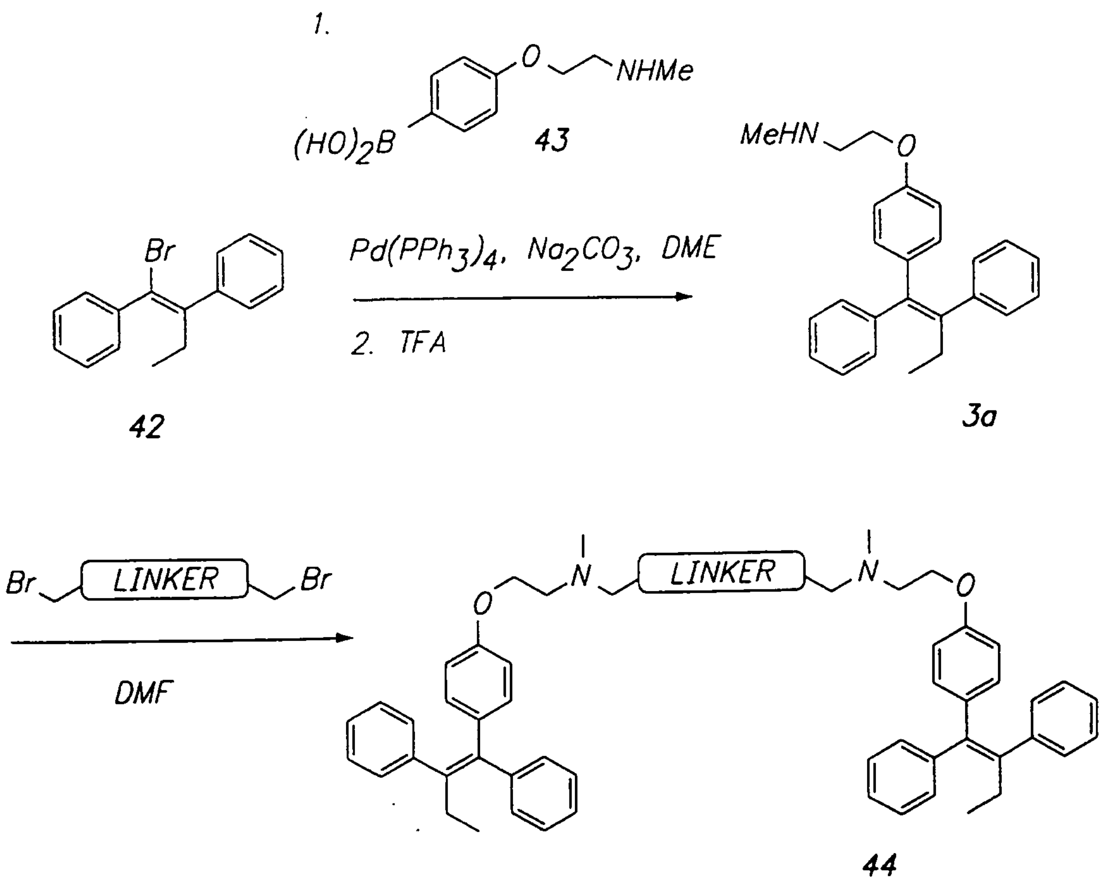
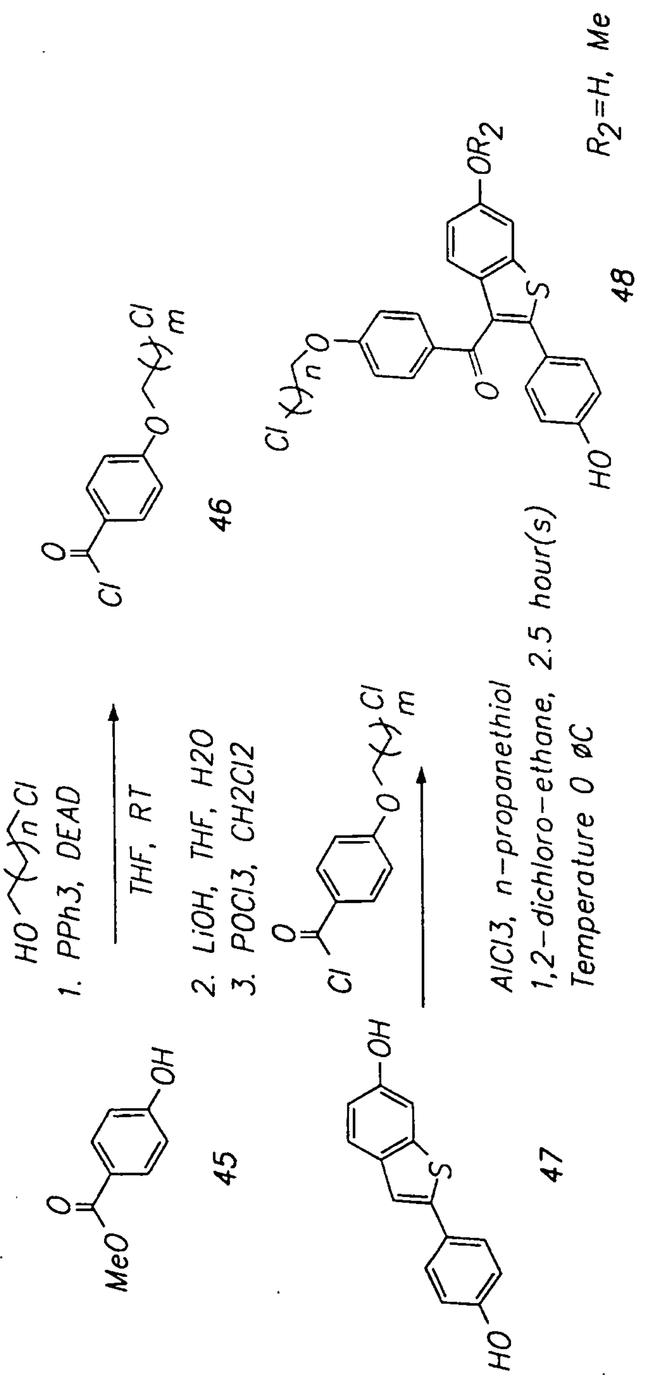

1.
NHMe
$(HO)_2B$
43
MeHN
Br
$Pd(PPh_3)_4$, Na_2CO_3, DME
2. TFA
42
3a
Br LINKER Br
DMF
LINKER
44

FIG. 14



MeO
OH
45
HO
1. PPh3, DEAD
THF, RT
2. LiOH, THF, H2O
3. POCl3, CH2Cl2
Cl
46
47
AlCl3, n-propanethiol
1,2-dichloro-ethane, 2.5 hour(s)
Temperature 0 øC
48
OR2
R2=H, Me

FIG. 15A

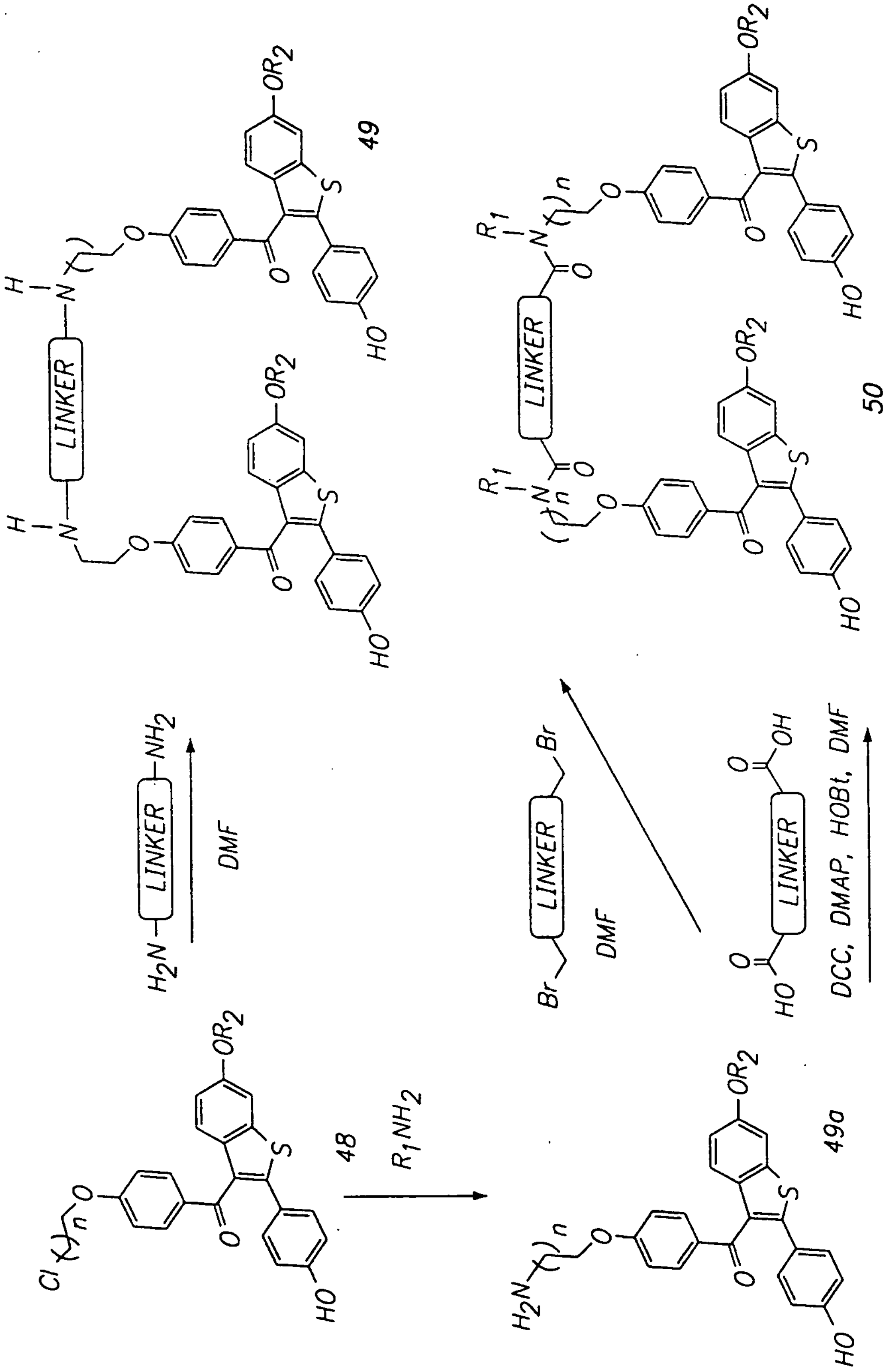
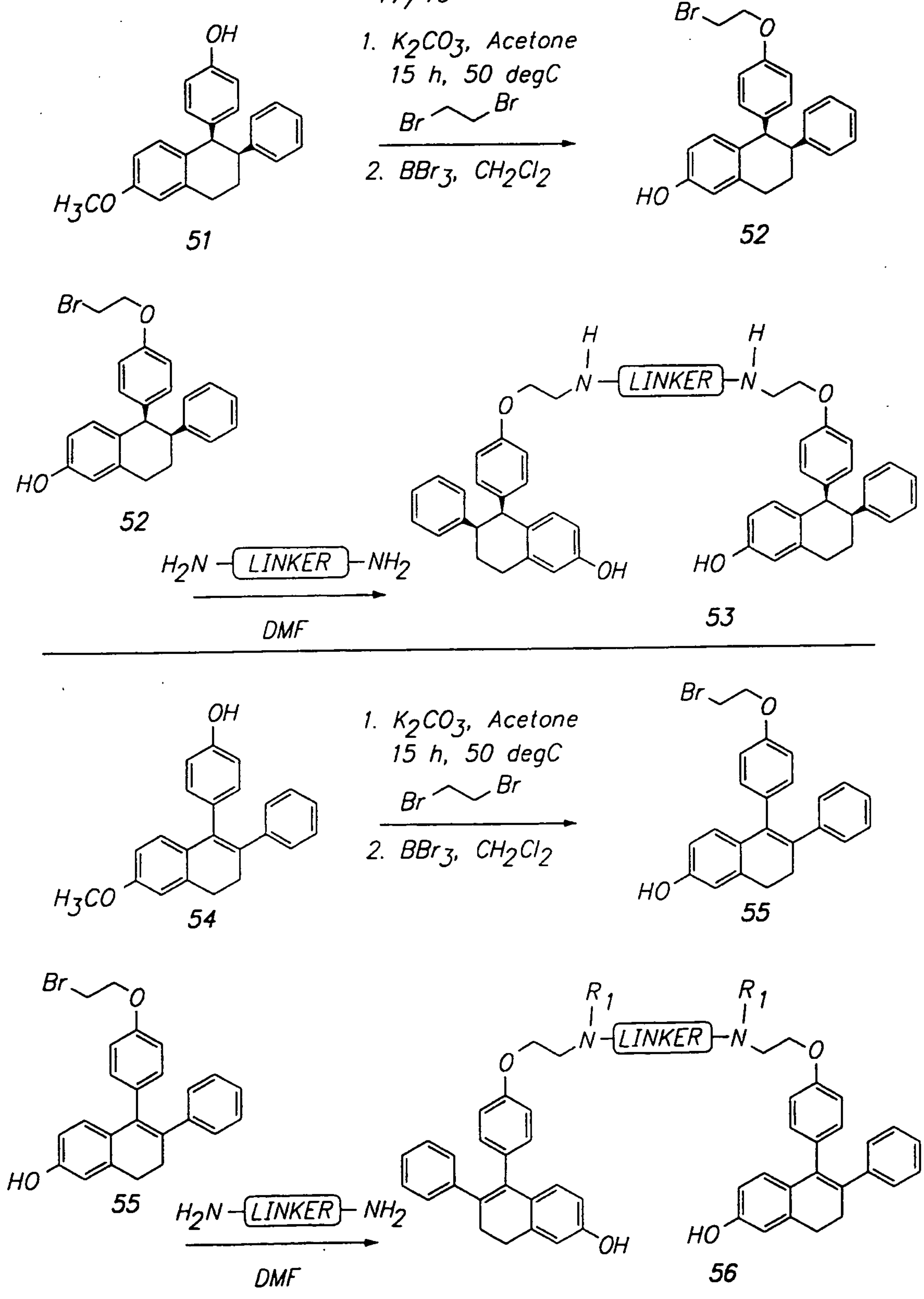

48
R_1NH_2
49a
H_2N LINKER NH_2
DMF
49
Br LINKER Br
DMF
HO LINKER OH
DCC, DMAP, HOBt, DMF
50

FIG. 15B



1. K_2CO_3, Acetone
15 h, 50 degC
Br Br
2. BBr_3, CH_2Cl_2
OH
H_3CO
51
Br O
HO
52
H N LINKER N H
O
OH
H_2N LINKER NH_2
DMF
53
54
55
R_1 N LINKER N R_1
56

FIG. 16

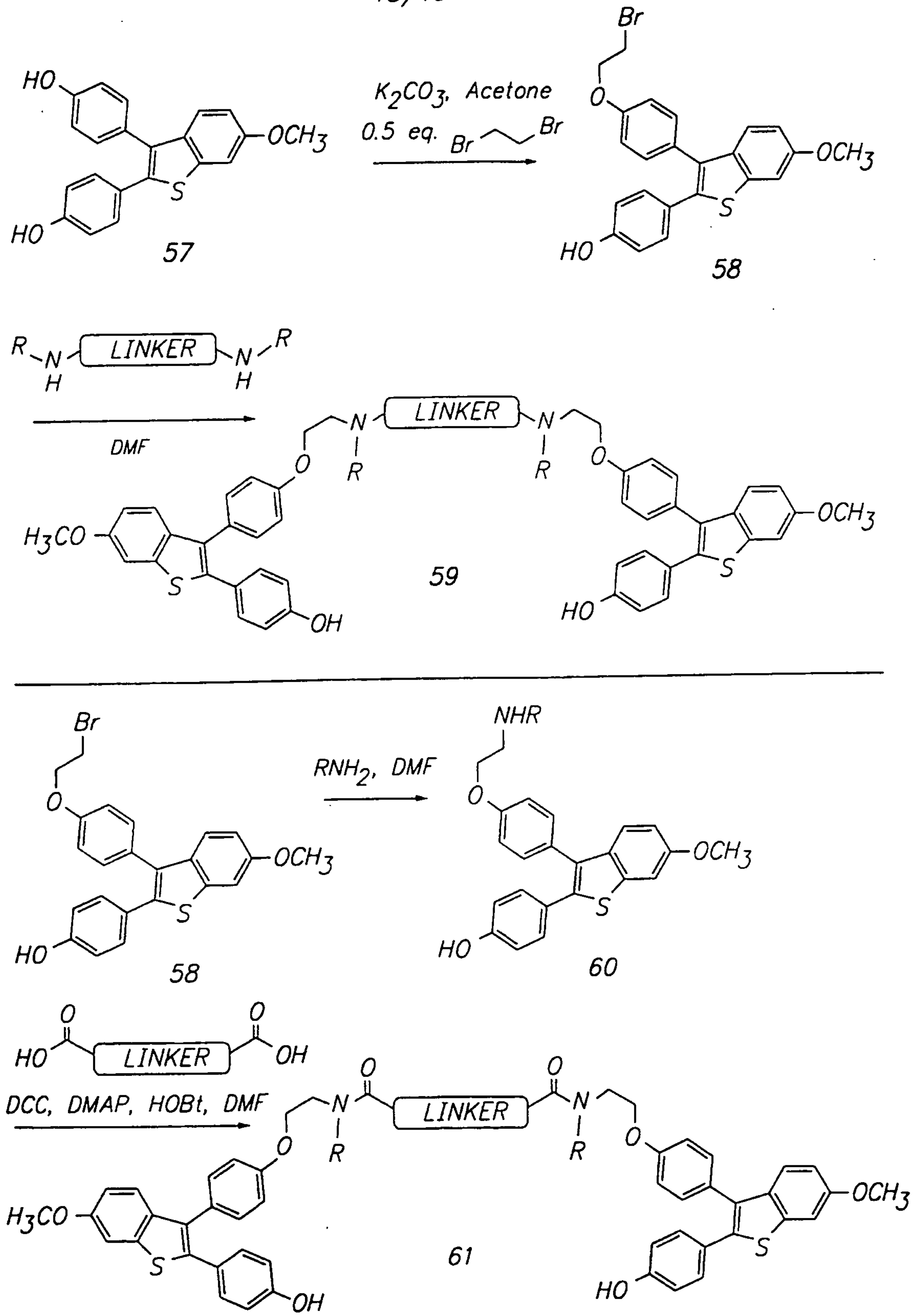
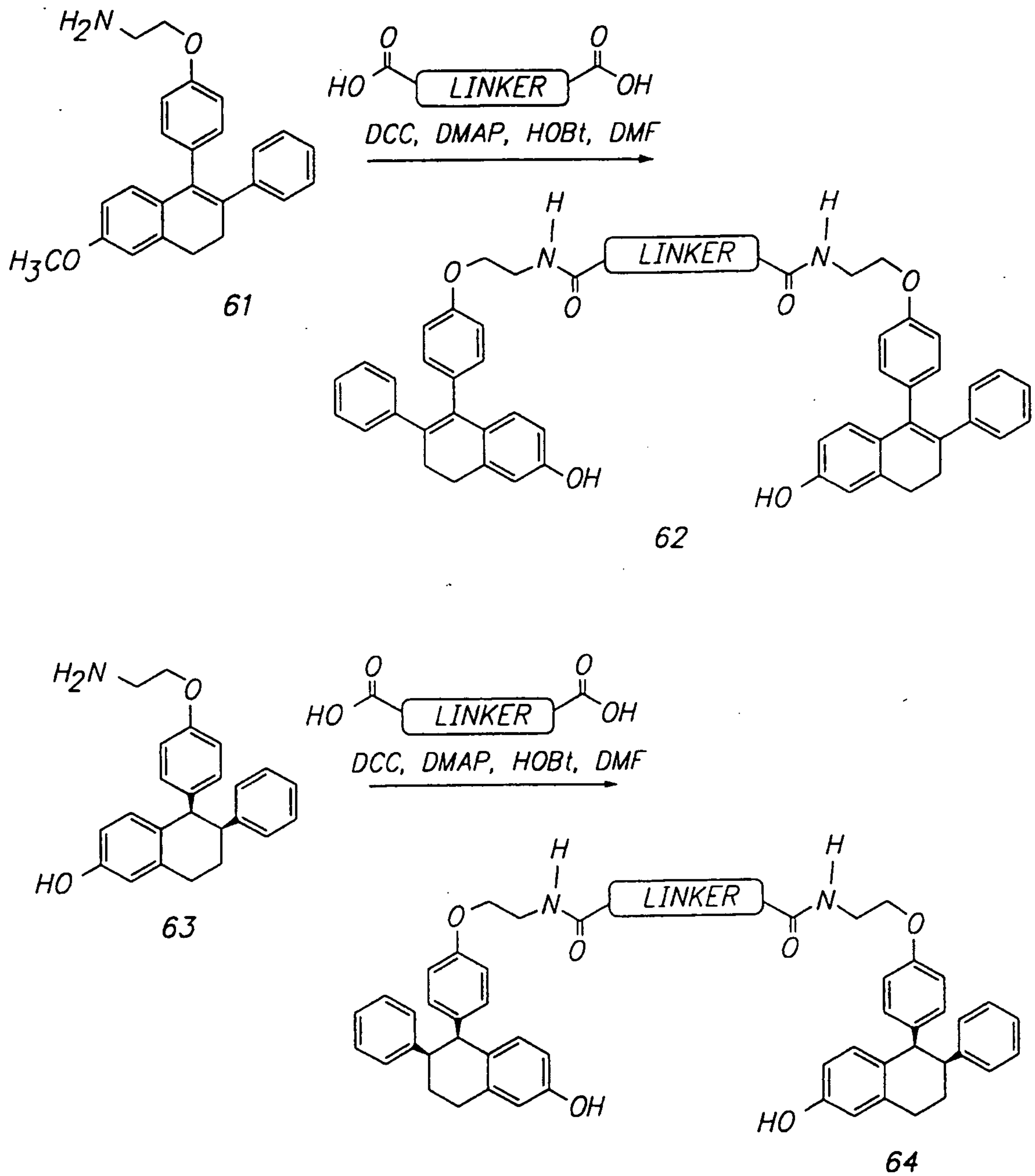

K2CO3, Acetone
0.5 eq. Br Br
57
58
LINKER
DMF
59
RNH2, DMF
60
DCC, DMAP, HOBt, DMF
61

FIG. 17



H2N
O
H3CO
61
O
O
HO
LINKER
OH
DCC, DMAP, HOBt, DMF
H
N
LINKER
H
N
O
O
O
O
OH
HO
62
H2N
O
HO
63
O
O
HO
LINKER
OH
DCC, DMAP, HOBt, DMF
H
N
LINKER
H
N
O
O
O
O
OH
HO
64

FIG. 18

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : A61K 31/42, 31/535, 38/00, 38/02, 38/16, 39/00, 39/44, 39/395, 51/00, C07D 265/30, 271/06, C07K 2/00, 4/00, G01N 33/53, 33/543, 33/566

A3

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Filed on	8 June 1998 (08.06.98)
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(71) Applicant (*for all designated States except US*): ADVANCED MEDICINE, INC. [US/US]; 280 Utah Avenue, South San Francisco, CA 94080 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): MORAN, Edmund, J. [CA/US]; 131 Chaves, San Francisco, CA 94127 (US). NUMEROF, Robert, P. [US/US]; Apartment 4, 140 Graystone Terrace, San Francisco, CA 94114 (US). GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US).

(74) Agents: SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313–1404 (US).

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(54) Title: NOVEL THERAPEUTIC AGENTS THAT MODULATE ESTROGEN RECEPTORS

(57) Abstract

Disclosed are novel multi-binding compounds (agents) which bind estrogen receptors. The compounds of this invention comprise a plurality of ligands each of which can bind to such receptors thereby modulating the biological processes/functions thereof. Each of the ligands is covalently attached to a linker or linkers which may be the same or different to provide for the multi-binding compound. The linker is selected such that the multi-binding compound so constructed demonstrates increased modulation of the biological processes mediated by the estrogen receptors.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12995

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BERGMANN et al. Bivalent Ligands as Probes of Estrogen Receptor Action. J. Steroid Biochem. Molec. Biol. June 1994, Vol. 49, No. 2/3, pages 139-152. See entire article, especially pages 139-140 and Table 1.	1-86
Y	HARDCASTLE et al. 4'-Substituted Analogues of Idoxifene: Antiestrogens and Calmodulin Antagonists. Bioorg. Med. Chem. Lett. 20 April 1995, Vol. 5, No. 8, pages 805-808. See entire article, especially page 805.	1-86
Y	US 5,681,835 A (WILLSON) 28 October 1997 (28.10.97), see entire document.	1-86

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 NOVEMBER 1999	07 DEC 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MAURIE E. GARCIA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	KLINGE et al. Comparison of Tamoxifen Ligands on Estrogen Receptor Interaction with Estrogen Response Elements. Mol. Cell. Endocrin. 25 August 1998, Vol. 143, pages 79-90. See entire article, especially Abstract and page 88.	1-86
Y, P	US 5,876,946 A (BURBAUM et al) 02 March 1999 (002.03.99), see Abstract and column 3 line 35 though column 7 line 25.	52-86
Y	US 4,910,152 A (MEYERS et al) 20 March 1990 (20.03.90), see Abstract, column 9 lines 3-28 and column 13 line 67 through column 15 line 25.	1-86
Y	WO 98/19162 A1 (NOVALON PHARMACEUTICAL CORPORATION) 07 May 1998 (07.05.98), see entire document, especially Abstract and pages 37-50.	52-86
Y	WO 92/05802 A1 (NEORX CORPORATION) 16 April 1992 (16.04.92), see Abstract, page 3 lines 1-25, page 4 lines 20-27, page 5 lines 6-18, page 21 lines 4-33, page 22 lines 1-8 and claim 1.	1-86
Y	WO 98/03632 A1 (YALE UNIVERSITY) 29 January 1998 (29.01.98), see entire document, especially pages 1-4 and 12-13.	1-86
Y	WO 97/35195 A1 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 25 September 1997 (25.09.97), see page 3 lines 17-32, page 4 lines 1-18, page 7 lines 26-34 page 8 lines 1-5 and claims 13, 35 & 36.	52-86
Y	SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	52-86

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12995

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/42, 31/535, 38/00, 38/02, 38/16, 39/00, 39/44, 39/395, 51/00; C07D 265/30, 271/06; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.1, 7.2; 436/501, 504, 518, 545; 424/1.11, 9.1, 178.1, 193.1; 514/237, 364, 520, 532, 570, 562, 599; 530/345, 389.1, 401, 807; 562/491, 558/401

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/7.1, 7.2; 436/501, 504, 518, 545; 424/1.11, 9.1, 178.1, 193.1; 514/237, 364, 520, 532, 570, 562, 599; 530/345, 389.1, 401, 807; 562/491, 558/401

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, STN (REGISTRY, EMBASE, MEDLINE, CAPLUS, SCISEARCH, BIOSIS)
Search terms: Structure search, estrogen receptor, ligand, multibinding, multivalent, polyvalent, bivalent, multimer, link?, bind?, combinatorial

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Species 1: 1st compound of claim 7
Species 2: 2nd compound of claim 7
Species 3: 3rd compound of claim 7
Species 4: 4th compound of claim 7
Species 5: 5th compound of claim 7
Species 6: 6th compound of claim 7
Species 7: 7th compound of claim 7
Species 8: 8th compound of claim 7
Species 9: 9th compound of claim 7
Species 10: 10th compound of claim 7
Species 11: 11th compound of claim 7

Note: compounds numbered left to right, then down the page.

The claims are deemed to correspond to the species listed above in the following manner: Some claims correspond only in part.

Species 1: 5, 7, 22, 24, 39, 41
Species 2: 5, 7, 22, 24, 39, 41
Species 3: 5, 7, 22, 24, 39, 41
Species 4: 5, 7, 22, 24, 39, 41
Species 5: 5, 7, 22, 24, 39, 41
Species 6: 6, 7, 23, 24, 40, 41
Species 7: 5, 7, 22, 24, 39, 41
Species 8: 5, 7, 22, 24, 39, 41
Species 9: 5, 7, 22, 24, 39, 41
Species 10: 5, 7, 22, 24, 39, 41

Species 11: 5, 7, 22, 24, 39, 41

The following claims are generic: 1-4, 8-21, 25-38, 42-86.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

PCT Rule 13.2 states that unity of invention shall be fulfilled when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features". It further defines "special technical feature" as "those technical features that define a contribution which each of the claimed inventions, claimed as a whole, makes over the prior art". For example, unity of invention is fulfilled if:
(a) all alternatives have a common property; and
(b) (i) a common structure is present, i. e. a significant structural element is shared by all alternatives, or
(b) (ii) in cases where the common structure can not be the unifying criterion, all alternatives belong to a recognized class of compounds in the art to which the invention pertains. (MPEP Section 1850).

In the instant case, part (a) above is fulfilled because all claimed species of ligand have a common property. However, the compounds encompassed by the instant formulas do not all possess a common structure (no shared significant structural element). Further, all of the species do not belong to a recognized class of compounds in the art to which they pertain. For the forgoing reasons, election under these rules is proper and required.